MUSCLE FUNCTION AND STRUCTURE IN SUBJECTS WITH IMPAIRED GLUCOSE TOLERANCE AND PATIENTS WITH TYPE 2 DIABETES IN RELATION TO VITAMIN D DEFICIENCY AND NEUROPATHY

A thesis submitted to The University of Manchester for the degree of

Doctor of Philosophy in Medicine

In the Faculty of Biology, Medicine and Health

2016

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THESIS ABSTRACT

Detailed assessment of motor function in patients with type 2 diabetes (T2DM) is important to identify early sub-clinical deficits and to implement early interventions in order to limit the development of advanced pathology and improve their quality of life. Lower limb muscular dysfunction has been demonstrated in several studies in patients with diabetic neuropathy (DN). Vitamin D deficiency is very common in patients with T2DM, both in subjects with impaired glucose tolerance (IGT) and in healthy subjects. Vitamin D deficiency can cause reduction in muscle strength, size, walking speed, a disturbance in dynamic sway during walking and an increased risk of falls in healthy elderly subjects. This project aimed to investigate muscle function, structure and kinematic alterations during walking activity in subjects with IGT and patients with T2DM in relation to vitamin D deficiency and neuropathy.

The work in thesis shows that diabetes is associated with reduced muscle strength and size of the lower extremities in patients with T2DM, and that is related to the severity of neuropathy but not vitamin D deficiency. Furthermore, small fibre neuropathy has been related to distal muscle weakness and increased dynamic medio-lateral sway during walking in subjects with IGT and patients with T2DM. Vitamin D deficiency has been related to muscle weakness in subjects with IGT but not to kinematic alterations during walking.
DECLARATION

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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CONTRIBUTION

The purpose of this section is to confirm that Monirah Almurdhi, the author of this thesis, was actively involved and made a significant contribution in all chapters/studies presented and discussed in this thesis. Initially, she undertook a pilot study in healthy control subjects to become expert in the study procedures prior to commencing the study in patients with T2DM and subjects with IGT. She recruited the majority of patients with T2DM, subjects with IGT and healthy controls to participate in the current study. She established lower limb muscle function and structure assessments which included active muscle strength assessment of the lower extremity and muscle size assessment using an MRI scanner, and she analysed all participants’ leg images. She calculated intramuscular fat within each muscle of the lower extremity from the MRI images. She assessed bone mineral density (BMD) for all the participants. In addition, she established the optimal protocol for gait analysis test and analysed their walking features to quantify walking variables for all study subjects. She collected and collated data from the above studies and performed all the statistical analysis to identify differences between study groups in the current thesis. Finally, she wrote all the chapters of this thesis and these were reviewed by her group supervisors: Professor Rayaz Malik, Professor Neil Reeves (Manchester Metropolitan University) and Dr Maria Jeziorska. Other tasks performed by the research team included:

- Patient recruitment performed by Mr Georgios Ponirakis and Professor Rayaz Malik.
- Electrodiagnostic studies performed by the consultant neurophysiologist Dr Andrew Marshall.

- Peripheral neuropathy assessments were performed by Drs Shazli Azmi, Hassan Fadavi, Uazman Alam and Omar Asghar.

- Ophthalmic examinations were performed by Drs Ioannis Petropoulos, Mitra Tavakoli and Maryam Ferdousi.

- Skin biopsy assessment was performed by Dr Maria Jeziorska.

- Blood and urine sample collections were undertaken by the nursing staff at the Wellcome Trust Clinical Research Facility.

- Haematology, immunology and clinical biochemistry analysis was performed and reported by the relevant departments under the directorate of Laboratory Medicine, Central Manchester University Hospitals, NHS Foundation Trust, UK.

- Vitamin D laboratory analyses were undertaken by the vitamin D laboratory at the Central Manchester Foundation Trust under the guidance of Dr Jacqueline Berry.
ALTERNATIVE THESIS FORMAT

The author, working within the Faculty of Biology, Medicine and Health, University of Manchester, has been granted permission by her supervisor Professor Rayaz Malik to submit her PhD thesis in alternative format approved under the regulations, including sections which are in a format suitable for submission for publication or dissemination.

The following list shows the chapters that accepted or submitted for publication: (for details see page 23).


- Chapter 4: Published in the journal Diabetes Care, January 2016.

- Chapter 5: Accepted for publication in the journal Diabetic Medicine, April 2016.

- Chapter 6: Submitted to the journal Diabetic Medicine, May 2016.
In the name of Allah, the beneficent, the merciful

“Perseverance is the pathway to success
and patience is the key to be strong and achieve your goal”

Dedication

This thesis is dedicated to my beloved parents.
They are the most important inspiration in my life, who have always
encouraged me and have trust in my abilities.

And also to my husband and my children who have supported me and been
patient and proud of me.
ACKNOWLEDGEMENTS

All hardwork comes to an end but there are always a series of difficulties which it presents. I cannot believe that I have finished my PhD study. However, behind this achievement there were some helpful individuals who my gratitude should go to; without them I would not have completed this scientific journey. It is really difficult to thank them all in a few lines.

First of all from the depth of my heart, truthful gratitude goes to Almighty ALLAH.

A big thanks and special gratitude and appreciation must go to my supervisor Professor Rayaz Malik who has been a very helpful and friendly supervisor for me throughout my work. Honestly, I have been so lucky to be one of his students. He has been an efficient source of continuous support and personal encouragement; without his motivation this piece of work would not have been created.

I would like to offer my thanks to my other supervisor Professor Neil Reeves from Manchester Metropolitan University for his guidance, support and expertise. He has spent much time and a great deal of effort to help me to understand and apply muscle function, structure assessment and image analysis. He also provided me the opportunity to have access to the muscle function laboratory at Manchester Metropolitan University and the library. I would like to express my thanks to my supervisor Dr Maria Jezierska for her assistance, guidance, comments and time. I would like to thank my co-supervisor Dr Frank Bowling for
his support and time as well as my advisor Professor Andrew Boulton for his help, comments and encouragement during my PhD.

I would like to thank my close friends and colleagues, Dr Maryam Ferdousi, Dr Shazli Azmi, Dr Ioannis Petropoulos, Dr Hassan Fadavi, Mr Georgios Ponirakis and Dr Andrew Marshall for their help, time, encouragement and expertise throughout this study. I would like to express my special thanks to Dr Steve Brown who had an important impact in helping me to understand muscle strength, gait assessment and analysis of walking variables using the Vicon nexus system, and also for his expertise and time. I would like to thank Dr Milos Petrovic for his help, encouragement and time.

I would like to acknowledge all the muscle function laboratory team at Manchester Metropolitan University and nursing staff at the Wellcome Trust Clinical Research Facility and all the subjects who have participated in this project. Without their contribution it would not have been possible to complete this work.

Last but not least, I would like to offer my special thanks to my husband Mr Ayaf who has been the source of my strength in every step of my way. He supported, encouraged and motivated me throughout my work. I am grateful to my parents for supporting me as well as my beautiful sisters Noora, Bedor and Manal and my brothers. I am very pleased to offer my thanks to my son Othman and my two lovely daughters Rawan and Joud who have been proud of me and helped me throughout my study.
PREFACE

Monirah Almurdhi graduated from the Applied Medical Sciences College, Faculty of Rehabilitation, King Saud University, Riyadh, Saudi Arabia and was awarded a Bachelor degree of applied medical sciences in the field of rehabilitation-physical therapy in 1997. She completed a one full-year internship in four different hospitals in Saudi Arabia from 1997 to 1998. She worked as a lecturer at the Rehabilitation Department between 1999 and 2010, and undertook theoretical and practical training lectures in the Applied Medical Sciences College and many hospitals in Riyadh, Saudi Arabia. Monirah has experience of academic teaching and clinical research and has been actively involved in research since the fourth year of her Bachelor degree. Monirah has always been interested in diabetes mellitus and neuropathy and developed her knowledge in this field. She studied for a MSc degree in neurology in physical therapy at Applied Medical Sciences College, King Saud University, Riyadh, Saudi Arabia from 2005 to 2009 where she undertook her research project on the “Effectiveness of Whirlpool Therapy in Diabetic Peripheral Neuropathy”. She studied for her MSc whilst working as a lecturer at the Rehabilitation-Physical Therapy Department. She continued her work for academic and social purposes and she was actively involved as a member of many student committees. She obtained her full scholarship to study for a PhD at the University of Manchester, Manchester, United Kingdom in 2011. In September 2012, she joined the cardiovascular research group at the School of Medical Sciences, University of Manchester, UK to begin her PhD in “Muscle function and structure in subjects with impaired glucose tolerance and patients
with type 2 diabetes in relation to vitamin D deficiency and neuropathy” under the supervision of Professors Rayaz A Malik, Neil Reeves, Andrew Boulton, Drs Maria Jezierska and Frank Bowling.

During her PhD, she presented part of this work in regional and international conferences between 2014 and 2016, and she published two papers from this work during 2016 in high impact factor journals.
LIST OF PUBLICATIONS:


2. **Almurdhi M**, Reeves N, Bowling F, Boulton A, Jeziorska M, Malik R. Distal Lower Limb Strength is Reduced in Participants with impaired Glucose Tolerance and is Related to Increased Intramuscular Fat and Vitamin D Deficiency. Accepted for publication in *Diabetic Medicine*, 2016. (Chapter 5)

3. **Almurdhi M**, Brown S, Bowling F, Boulton A, Jeziorska M, Malik R, Reeves N. Altered walking strategy and increased unsteadiness in subjects with impaired glucose tolerance and type 2 diabetes relates to small fiber neuropathy but not vitamin D deficiency (Submitted for publication to *Diabetic Medicine*, May 2016). (Chapter 6)

LIST OF ABSTRACTS:

   Lower limb muscle function and structure in patients with type 2 diabetes in relationship to neuropathy, intramuscular fat and vitamin D levels. 9th Saudi Student Conference in UK 13-14 February 2016, Birmingham, United Kingdom (Oral Presentation).

   Reduced lower limb muscle strength and volume in patients with Type 2 diabetes: Relationship to neuropathy, intramuscular fat and vitamin D levels. Neurodiab 2015 annual meeting 11-13 September 2015, Elsinore, Denmark (Poster Presentation).


   Vitamin D deficiency in relation to neuropathy and muscle function in diabetic patients. 8th Saudi Student Conference in UK 31 January-01 February 2015, London, United Kingdom (Poster Presentation).

   Muscular dysfunction in patients with type 2 diabetes. 7th Saudi Student
Conference in UK 01-02 February 2014, Edinburgh, United Kingdom (Poster Presentation).
LIST OF ABBREVIATIONS

$1,25(\text{OH})_2\text{D}$: 1,25-dihydroxyvitamin D

$25(\text{OH})\text{D}$: 25-hydroxyvitamin D

BF: Biceps femoris

BMD: Bone mineral density

BMI: Body mass index

BSA: Body surface area

CCM: Corneal confocal microscopy

CI: Confidence interval

CIP: Cold induced pain

CNBD: Corneal nerve branch density

CNFD: Corneal nerve fibre density

CNFL: Corneal nerve fibre length

CNFT: Corneal nerve fibre tortuosity

CoM: Centre of mass

CoP: Centre of pressure

CSA: Cross sectional area

CT: Cold threshold

DCCT: Diabetic control and complications

DEXA: Dual energy x-ray absorptiometry
DF: Dorsiflexors

DN: Diabetic neuropathy

DPN: Diabetic peripheral neuropathy

DSPN: Diabetic sensorimotor polyneuropathy

GLUT: Glucose transporter

HbA1c: Glycated Haemoglobin A1c

HDL: High density lipoprotein

ICC: Intra-class correlation coefficient

IENFD: Intraepidermal nerve fibre density

IFG: Impaired fasting glucose

IGT: Impaired glucose tolerance

IMAT: Intramuscular adipose tissue

IMNCT: Intramuscular non-contractile tissue

IRM: Institute for biomedical research into human movement and health

IVCCM: In vivo corneal confocal microscopy

LDL: Low density lipoprotein

LG: Lateral head of gastrocnemius

MG: Medial head of gastrocnemius

MNCV: Motor nerve conduction velocity

MRI: Magnetic resonance imaging

MVC: Maximal voluntary contraction
NC: Nerve conduction

NCV: Nerve conduction velocity

NDS: Neuropathy disability score

NHS: National Health Service

OGTT: Oral glucose tolerance test

PKC: Protein kinase C

PMNA: Peroneal motor nerve amplitude

PMNCV: Peroneal motor nerve conduction velocity

PTH: Parathyroid hormone

RF: Rectus femoris

SD: Standard deviation

SM: Semimembranosus

SNAP: Sural nerve amplitude

SNCV: Sural nerve conduction velocity

SOL: Soleus

ST: Semitendinosus

T1DM: Type 1 diabetes mellitus

T2DM: Type 2 diabetes mellitus

UKPDS: United Kingdom prospective diabetes study

UVB: Ultraviolet radiation B

VDR: Vitamin D receptor
**VI:** Vastus intermedius

**VL:** Vastus lateralis

**VM:** Vastus medialis

**VPT:** Vibration perception threshold

**WIP:** Warm induced pain

**WT:** Warm threshold
1 CHAPTER I. INTRODUCTION
1.1 Diabetes mellitus

Diabetes mellitus is an epidemic metabolic disease characterized by an inability of the body to produce and/or resist insulin action leading to hyperglycaemia. It includes two main types: Type 1, when insulin production is reduced or abolished; Type 2, when initially insulin resistance occurs followed by inadequate production (1, 2). According to the World Health Organization, 347 million people are thought to currently suffer from diabetes in the world (3). However, the most recent International Diabetes Federation figures estimate that there are in fact 382 million people with diabetes, 316 million people with impaired glucose tolerance and a predicted 552 million people who will have diabetes by 2030 (4).

Whilst the major cause of morbidity and mortality is related to macrovascular and microvascular complications, of note is that diabetes is a major risk factors for falls (3). Given that the population is ageing and that one in three community-dwelling 65 year-olds will suffer from one or more falls annually (5), this represents a major morbidity in terms of hip fracture and subsequent increased mortality (6). Moreover, vitamin D deficiency is highly prevalent in patients with type 2 diabetes (T2DM) (7, 8) and in subjects with impaired glucose tolerance (IGT), and has been related to hypertension, cardiovascular problems, obesity and diabetes (9, 10). With regard to subjects with impaired fasting glucose (IFG) and/or IGT, although the progression rate to diabetes is ~7% annually, IGT per se represents a major health problem and is an important risk factor for macrovascular disease (3).
1.1.1 Diagnosis of diabetes mellitus

Diabetes can be diagnosed from plasma glucose concentration following an oral glucose tolerance test, taking into account the fasting and 2-hour glucose levels following ingestion of a 75g oral glucose solution (Table 1-1).

Table 1-1. Diagnosis of diabetes mellitus.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plasma glucose concentration (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impaired fasting glucose</td>
<td>Fasting ≥6.1 and &lt;7.0 and/or 2-hours post glucose load &lt;7.8</td>
</tr>
<tr>
<td>Impaired glucose tolerance</td>
<td>2-hour post glucose load ≥7.8 and &lt;11.1</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>Fasting ≥7.0 and/or 2-hours post glucose load ≥11.1</td>
</tr>
</tbody>
</table>

Glucose Load= 75g of glucose dissolved in water taken orally (OGTT= oral glucose tolerance test) (2, 3, 11).

1.1.2 Pathogenesis of type 2 diabetes mellitus

β-cell dysfunction and insulin resistance are the most important factors in the pathogenesis of T2DM. That genetics plays a role is clear from observations that impaired glucose tolerance/diabetes occurs in 15-25% of subjects when both parents have T2DM and from the fact that prevalence of T2DM was 38% at 80 years of age if one parent had T2DM but was 60% by the age of 60 years if both parents had T2DM (3). However, no definitive causal gene has been identified to date and this has hindered efforts to clearly identify functional candidate genes and interpret their role in mediating susceptibility to diabetes (12).
1.1.2.1 Pathophysiology of hyperglycaemia

Insulin is central to the control of blood glucose concentrations, and balanced insulin secretion and action are fundamental to maintaining normoglycaemia. Secretion of insulin by β-cells can normalize or reduce hepatic glucose production, inhibit fatty acid secretion by adipose tissue and increase glucose uptake by skeletal muscles. Thus, β-cell dysfunction is an essential factor in the pathogenesis of IGT and T2DM. However, insulin resistance results in reduced glucose uptake by skeletal muscles and increased glucose and fatty acid production by the liver, which further impairs insulin secretion and action in patients with T2DM and subjects with IGT (Fig.1-1). Furthermore, obesity and reduced physical activity have been strongly associated with insulin resistance. Increased storage of triglycerides in the viscera or deep subcutaneous adipose and non-adipose cells, e.g. intramyocellular lipids, can enhance insulin resistance in skeletal muscle and liver (Fig.1-1) (3). Vitamin D deficiency may be an important risk factor for T2DM beyond abnormalities in body mass index (BMI), insulin resistance and β-cell dysfunction (9, 13). Thus, vitamin D metabolism may play an important role in the pathogenesis of T2DM (9).
Figure 1-1. Pathophysiology of hyperglycaemia and increased fatty acids in T2DM (3).

1.2 Impaired glucose tolerance

Pre-diabetes or IGT is an intermediate stage of hyperglycaemia (14) which is diagnosed when plasma glucose is 7.8-11.1 mmol/L (140-200 mg/dL) 2-hours after the ingestion of 75g of oral glucose (Table 1-1) (2, 3, 11) or when haemoglobin A1c (HbA1c) is between 5.7% and 6.4% (2).

1.2.1 Prevalence of impaired glucose tolerance

The prevalence of IGT globally was estimated to be 343 million (7.8%) in 2010, ranging from 5.8% in South East Asia to 11.4% in North American and Caribbean countries. The International Diabetes Federation predicts an increase, with the number of cases to reach 471 million across the world by 2035 (14).
1.3 Diabetic neuropathy

Diabetic neuropathy (DN) is the most common long-term complication in patients with diabetes, resulting in sensory, autonomic and motor nerve impairment with predominantly symmetric features although they can be asymmetric (15). The classification of DNs differs according to the aetiology, pathology, symptoms, clinical features and pattern of nerve damage (16). DN is classified into three main types: generalised neuropathy, focal and multifocal. Generalised neuropathy is subdivided into three groups: chronic sensorimotor neuropathy, acute sensory neuropathy and autonomic neuropathy. Focal neuropathy is subdivided into two groups; cranial and focal limb neuropathy. Multifocal neuropathy includes radiculoplexus neuropathies and truncal radiculoneuropathy (5).

1.3.1 Diabetic polyneuropathy

Diabetic polyneuropathy (DPN) is a cause of high morbidity and mortality in patients with diabetes (16-18) and is defined as the existence of clinical symptoms and/or signs of peripheral nerve damage of the lower limbs, after excluding other causes of neuropathy (5). DN occurs in both types of diabetes but is more prevalent in T2DM (16) due to dysfunction of the peripheral nerves and distal muscles (19).
1.3.1.1 Prevalence of diabetic polyneuropathy

DPN occurs in 20 to 50% of all diabetic patients (5, 20-25). Even at the diagnosis of T2DM, around 7% have neuropathy (26), and 50% of diabetic patients aged over 60 (27, 28), with a diabetes duration of more than 25 years suffer from DPN (26). Furthermore, 30% of patients with diabetes are admitted to hospital due to complications related to DN and ~20% of community patients suffer from neuropathy (29). The Diabetic Control and Complications (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS) found that the incidence of neuropathy was approximately 2% each year in patients with diabetes (30). Studies in several European countries have shown a prevalence of DPN ranging from 23 to 29% (5, 31).

1.3.1.2 Risk factors for diabetic polyneuropathy

There are important variables which increase the susceptibility and severity of DPN such as age (29, 32), hypertension, higher levels of HbA1c, longer duration of diabetes, higher levels of lipids, smoking and obesity. In addition, obesity and peripheral arterial diseases can also result in the development of neuropathic pain (5, 24, 29, 32).

1.3.1.3 Severity of diabetic polyneuropathy

The severity of polyneuropathy can be identified using the Toronto criteria:
- Grade 0 = No abnormality of nerve conduction (NC) velocity.
- Grade 1a = Abnormality of NC, without symptoms or signs.
- Grade 1b = NC abnormality of stage 1a plus neurological signs typical of diabetic sensorimotor polyneuropathy (DSPN) but without neuropathy symptoms.
- Grade 2a = NC abnormality of stage 1a with or without signs (but if present <2b) and with typical neuropathic symptoms.
- Grade 2b = NC abnormality of stage 1a, a moderate degree of weakness (50%) of ankle dorsiflexion with or without neuropathy symptoms (33).

1.3.1.4 Pathogenesis of neuropathy

DN is considered to be a metabolic and microvascular disease. Indeed, hyperglycaemia in the cell leads to oxidative stress, protein glycation and increased polyol pathway metabolism. Poor glycaemic control is associated with DSPN (17). Microvascular complications are characterised by vasoconstriction (34), increased thickness of the capillary basement membrane and endothelial cell hypertrophy, and hyperplasia with luminal occlusion and endoneurial hypoxia (35-38).
1.3.1.4.1 Oxidative stress

Increased intracellular glucose concentration produces carbonyls which react with proteins or lipids to create glycoxidation and/or lipoxidation compounds and carbonyl complexes with proteins resulting in oxidative stress (39).

1.3.1.4.2 Advanced glycation end products

Normally, glucose and other sugars can non-enzymatically form covalent bonds with proteins through the Maillard reaction to produce Schiff bases and Amadori products which react to make advanced glycation end products (AGEs). However, when there is intracellular hyperglycaemia this leads to glycation of important proteins which impairs their structure and function. Increased AGEs have been related to microvascular dysfunction and the pathology of DN (40).

1.3.1.4.3 Protein Kinase C

Increased glucose concentration within the cell can cause increased diacylglycerol and protein kinase C (PKC) concentrations in retinal, aortic and renal tissues. However, surprisingly, neuronal concentrations of diacylglycerol and PKC may be unchanged or diminished in diabetes (41, 42). Despite this, it has been shown that PKC inhibitors may improve Na\(^+\) - K\(^+\)-ATPase activity and nerve conduction velocity (NCV) in patients with diabetes (41).
1.3.1.4.4 Polyol pathway

Generally, aldose reductase enzyme reduces toxic aldehydes within the cell to inactive alcohols. When the glucose concentration is too high, aldose reductase reduces glucose to sorbitol by oxidation of NADPH to NADP$^+$, and sorbitol is further oxidized by sorbitol dehydrogenase by reducing NAD$^+$ to NADH and consuming the cofactor NADPH to fructose (Fig.1-2) (43). NDAPH reduces glutathione reductase with increased susceptibility to oxidative stress (44, 45).

Figure 1-2. Pathogenesis of hyperglycaemia and increased polyol pathway mechanism (46).
1.3.2 Diabetic sensorimotor polyneuropathy

DSPN is a symmetric polyneuropathy which has been related to the duration of diabetes and chronic hyperglycaemia (5, 15). DSPN is characterised by impairment of large and small nerve fibres causing respectively large and small fibre neuropathy (5, 47, 48). Large fibre neuropathy is recognized by a deficit in vibration, light touch and joint position sense with loss of ankle reflexes (5, 18, 19, 47, 48). Small fibre neuropathy occurs earlier and is characterized by paraesthesia, dysesthesia, burning and tingling sensation, and cold or heat feelings (5, 18). The most frequent sites of pain are the feet (96%), balls of the feet (69%), toes (67%), dorsum of the foot (54%), hands (39%), base of the foot and calves (37%) and heels (32%) (5). Autonomic neuropathy is also commonly associated with distal neuropathy (47, 48).

A painful, sensory small fibre neuropathy can occur in subjects with IGT (14). Indeed, sensory deficits can occur at the diagnosis of T2DM and in subjects with IGT and can include motor problems (49-51).

Neuropathy can be diagnosed using nerve conduction velocity (NCV) (52), and therefore much of the focus has been on nerves. However, a loss of contractile function of the skeletal muscles has been related to increased non-enzymatic glycation (52-54) and impairment of balance during walking (47, 48).
1.4 Motor complications

Motor dysfunction can occur directly as a consequence of neuropathy and denervation of the foot muscles (15). Typically, motor dysfunction starts distally in the extremities, particularly in the lower limbs, and is characterised by quite marked muscle wasting but initially with preserved strength. Denervation, muscle weakness, muscle atrophy, tendon stiffness and a limited range of motion characterise skeletal muscle abnormalities (55, 56). The most common and prominent motor symptom, and the one that has the most impact on the quality of life of patients with diabetes mellitus is weakness of the distal knee (extensors and flexors) and ankle (dorsiflexors and plantar flexors) muscles with muscle weakness and atrophy (20, 57).

1.4.1 Foot structure abnormalities

Common structural alterations in the diabetic foot include excessive callus formation, protruding metatarsal heads, claw/hammer toe deformity, hallux valgus and limited joints mobility. As a result of these structural changes, a higher load pressure occurs in specific areas under the metatarsal heads which increases the risk of foot ulcers (18, 58, 59). Limitation of the range of motion at the ankle and first metatarso-phalangeal joints influences the normal walking pattern of the foot during the stance phase and increases plantar pressures in the forefoot and toes. Muscle atrophy is common but does not necessarily lead to foot deformity (59).
1.4.2 Muscle strength

1.4.2.1 Examination of muscle strength

Motor function can be evaluated by different methods:

1. Functional assessment is simple and does not require instruments. This can be done by testing a patient’s ability to walk on heels and toes, reflecting ankle plantar and dorsiflexor strength or the ability to stand from a kneeling position which reflects muscle strength of the proximal muscles. This method has many limitations for assessing muscle strength accurately. Limited studies have been done to investigate the exact relationship between functional assessment and muscle strength. There are many other factors that may also affect the ability to perform these functions such as age, muscle disuse etc. (60, 61). In addition, there are other factors that may impair the ability to stand on the heels like loss of proprioceptive sensation, loss of vision, pain, orthopaedic problems and limited ankle and toe joint mobility (15).

2. A manual muscle test is a standard and semiquantitative way to examine muscle strength but similar to the functional tests described above it has many limitations. It is very subjective and insensitive compared to quantitative assessments of muscle strength, particularly in the proximal muscles with a short lever arm (62). The assessment of ankle plantar
flexor muscle strength was not shown to be reliable using manual muscle strength tests (15). Manual muscle assessment of the knee extensors and flexors is not sensitive enough to adequately quantify muscle weakness compared to quantitative instruments such as the isokinetic dynamometer (15).

3. An isokinetic dynamometer is the most common way to objectively assess muscle strength in healthy people and across disease states (63, 64). Static (isometric) and dynamic (isokinetic) muscle strength data of all muscle groups of the upper and lower extremities can be provided using an isokinetic dynamometer (15). An isokinetic dynamometr is a sensitive and quantitative procedure for the assessment of mild to moderate weakness (15, 57). Muscle strength is measured as joint torque, and is most commonly measured at maximal isometric muscle contraction using a linear scale that is shown on the isokinetic dynamometer screen (15). Dynamic muscle strength testing can also be conducted in concentric (muscle shortening) and eccentric (muscle lengthening) modes using an isokinetic dynamometer. Although also accurately reflecting muscle strength capabilities, such dynamic measures in addition involve an element of motor control, which adds complexity for the neuromuscular system as it requires some learning (65). Therefore, especially in patients with disease, isometric muscle testing is likely the most optimal form of testing to accurately reflect muscle strength.
1.4.2.2 Muscle strength in healthy subjects

Muscle strength of different muscle groups in the upper and lower extremities of healthy subjects can be affected by age, gender, height and weight (15, 66). These differences in muscle strength and function could also be explained by genetic variations among healthy subjects (15, 67). In healthy subjects, muscle strength is maximal around the age of 30 years and then deteriorates with increasing age to the age of 60-65 years (15). Thus, a young male is ~five times stronger than an elderly female.

1.4.2.3 Muscle strength of the lower limb in patients with diabetes

Motor impairment is related to the severity of DN (15, 57). Distal muscle weakness has been related to the deficits in the motor nerves. The small intrinsic muscles of the feet and hands are particularly affected, followed by gross muscle weakness of the lower extremities. More extreme alterations such as Charcot foot and amputation of the lower limb as a consequence of DN (19, 48, 68) will clearly have a more profound impact on motor function. Both T1DM and T2DM patients suffer from weakness of distal muscle strength particularly at the knee (knee extensors and flexors) and ankle (ankle plantar and dorsiflexors) joints with decreased muscle function and altered daily activity (15, 57, 69). Muscle strength of the knee extensors is inversely correlated with insulin resistance in patients with T2DM with and without DSPN (70). Thus, patients with diabetes have lower
limb muscle weakness which relates to reduced physical activity and quality of life (15).

1.4.2.4 Molecular alterations

Skeletal muscles are composed of three different types of fibres: I (slow-twitch), IIa, and IIb (fast twitch) which vary according to the level of muscle activity. Furthermore, muscle plasticity can control the proportion of the muscle fibres (fast and slow twitch). The distribution and type of skeletal muscle fibres also play a role in insulin resistance via two glucose transporters GLUT1 and GLUT4, and the type of fibres as type I slow-twitch fibres are more sensitive to insulin than fast-twitch fibres (71). There is a higher percentage of GLUT4 in slow-twitch fibres compared to fast-twitch fibres and the amount of slow-twitch fibres declines significantly in T2DM. In subjects with normal glucose tolerance, GLUT4 is significantly increased in slow-twitch fibres, but in patients with T2DM, GLUT4 is much lower in slow-twitch fibres, contributing to insulin resistance (71).

1.4.3 Muscle atrophy of the lower limb

In parallel to the muscle weakness, atrophy of the distal skeletal muscles of the lower extremity can occur in advanced DSPN, starting in the distal muscles of the foot initially and then progressing to the proximal muscles of the leg (15). Muscle atrophy is characterized by a reduction in both muscle bulk and size with reduced
muscle bulk of extensor digitorum brevis, prominent metatarsal heads and claw
toes a typical characteristic of DN (15). There is a significant association between
motor NCV and muscle atrophy (18, 59). Muscle atrophy can be quantified using
MRI (magnetic resonance imaging) which is a radiological imaging technique that
can quantify muscle size and atrophy (15, 57). MRI is a non-invasive clinical
examination method which produces very high resolution images differentiating
muscles, bones, fat and connective tissue (72). However, MRI is expensive,
time-consuming and cannot be performed in patients with pacemakers and/or
metal devices (15).

Patients with DN have muscle atrophy in both the mid (43%) and distal (65%)
leg, and muscle volume is reduced by 32% in patients with DN compared to
healthy control subjects (55). In addition to muscle atrophy, there is increased
signal intensity which is indicative of fatty infiltration in the ankle plantar and
dorsiflexor muscles (69). Indeed, increased signal intensity has been related to
neuropathy as there was no increase in signal intensity or muscle atrophy
reported in patients without neuropathy compared to patients with neuropathy
(15). It has been suggested that increased fat tissue in and between skeletal
muscles in patients with diabetes is related to reduced aerobic capacity (56).

Other functional deficits such as muscle fatigue may occur in patients with T2DM
and can be identified using an isokinetic protocol. Fatigue in patients with T2DM
has been related to a higher percentage of type II fast-twitch fibres, with a greater
tendency to fatigue, and a lower percentage of type I muscle fibre which are less
prone to fatigue (20).
Motor dysfunction also includes limitations in the range of motion. This results in a reduction in daily living activities such as walking and climbing stairs (73, 74). A limitation in the range of motions occurs initially during dorsiflexion at the ankle joint and at the first metatarso-phalangeal joint, which is greater in neuropathic diabetic patients in comparison with non-neuropathic diabetic patients and healthy subjects (55, 57).

1.4.4 Diabetic sensorimotor polyneuropathy and gait

Sensory and motor dysfunction play an important role in abnormal gait and posture due to reduced vibration sense, tactile pressure and proprioception (22, 75). A disturbance in afferent and efferent fibres results in slowness of gait speed, widening and longer stance phase with shorter steps and a consequent increased risk of falls (20, 22, 27, 51, 55, 57, 74, 76-79). Increased tendon stiffness in the ankle and knee joints can impair walking activity in patients with diabetes with or without neuropathy (80-83). A fear of falls is a possible explanation for the slow walking speed in patients with diabetes (15). Diabetic patients also use the “hip strategy” to raise their leg by pulling the hip up and through the swing phase as an alternative to using the ankle plantar flexors and “ankle strategy” to propel the leg into the swing phase, which results in slower walking speed and shorter step length (83, 84). Thus, ankle plantar flexor muscle strength is a fundamental factor that walking activity depends on, and weakness and stiffness in this group of muscles may cause walking alterations in patients.
with diabetes (83, 84). Reduced distal muscle strength of the lower limb and peripheral neuropathy can deteriorate joint moments and muscle power production of the lower limb causing an alteration in gait performance (22, 56) and lower extremity physical function (21). Indeed, gait abnormalities can be detected before the onset of neuropathy in patients with diabetes (25, 78, 85, 86).

1.4.5 DSPN and disturbance of balance and falls

Patients with DN have an approximately 50% greater probability of falling annually compared to healthy control subjects (87, 88) which was approximately 15 to 23 times greater in patients with DN (89, 90). As previously discussed, sensory and motor deficits, reduced mobility and altered gait with an impairment in balance can lead to increased falls and severe injuries (22, 74, 85, 86, 91-94). This can be further worsened by a reduction in the visual field (95, 96), resulting in a disturbance of postural balance both during walking (22, 50, 76, 92) and whilst in the standing position (96-98). A disturbance in balance and trunk stabilization during quiet standing has been observed in subjects with IGT and neuropathy (50). The centre of mass (CoM) is positioned in the centre of the human body between L1 and L2 vertebrae and directed down towards the ground vertically, while centre of pressure (CoP) arises from the foot and is directed vertically upwards to the head. When the CoM is located over the CoP, the body is stable during standing (74). An increased separation distance
between these two variables may demand higher muscular effort to keep the body more stable during walking activities. It has been found that patients with diabetes have a greater step width and increased dynamic medio-lateral sway during walking which results in a disturbance of balance (74). Indeed, this higher separation between CoM and CoP during walking may explain why patients with diabetes are at high risk of falling. Postural instability with a larger centre of pressure displacement and higher dynamic sway area particularly in the medio-lateral direction has been associated with sensory neuropathy and an increased risk of falls (96, 99). Thus, DSPN and biomechanical changes that cause foot problems may play an important role in the disturbance to balance during standing and an increased risk of falls (100). Motor dysfunction is complex and can cause significant walking and balance impairment with an increased possibility of falls in patients with DSPN (15).

1.5 Diabetes and vitamin D deficiency

Patients with both T1DM and T2DM have lower levels of vitamin D than non-diabetic subjects (101-103). 89.2% of patients with diabetes have vitamin D deficiency compared to 75% of healthy control subjects with vitamin D deficiency (103). In another study, 98.4% of patients with T2DM with HbA1c>7% had insufficient levels of vitamin D (<50nmol/L) compared to those diabetic patients with HbA1c≤7% (100%); the researchers found it did not correlate with glycaemic control (8). Conversely, an Asian population with vitamin D deficiency had a higher prevalence of T2DM (3.4 times) compared to those with normal vitamin D
levels (9). Thus, there appears to be a link between diabetes and vitamin D deficiency but the exact mechanism is not known (9). Both diabetes mellitus and vitamin D deficiency are common diseases. With regard to vitamin D deficiency, although there is a wide range in different geographical areas and with different ages, low levels of 25-hydroxyvitamin D (25(OH)D) have been found in patients with T2DM (104) and subjects with IGT (9). Vitamin D deficiency may mediate its effects on glucose levels through altered calcium dependent insulin release and transport and utilisation of glucose during muscle contractions (105). Furthermore, vitamin D deficiency contributes to increased parathyroid hormone (PTH) which may increase circulating fatty acids. This, in turn, may result in increased insulin resistance (106). Indeed, insulin secretion is reduced in vitamin D deficiency and replacement of vitamin D improves β-cell function and glucose tolerance (7). Patients with diabetes and inadequate levels of vitamin D have lower physical function compared to non-diabetic patients with adequate vitamin D levels (107), which may further contribute to reduced glucose utilisation.

Of course, low levels of vitamin D can lead to osteomalacia and proximal myopathy due to a disturbance in calcium handling (7, 108) as well as sarcopenia with a reduction in skeletal muscle strength and endurance (109).

1.5.1 Vitamin D

Evidence of evolutionary preservation is confirmed by the fact that a phytoplankton species, “emiliania huxleyi”, which resides in the Atlantic Ocean
and has apparently not changed for more than 750 million years, was found to generate vitamin D when exposed to sunlight (110).

There are two main types of vitamin D: ergocalciferol or vitamin D2 which exists in yeast and plants after ultraviolet radiation, and cholecalciferol or vitamin D3 which can be synthesised by exposure of human skin to UVB radiation. Cholecalciferol is hydroxylated twice to produce 1,25-dihydroxyvitamin D (1,25(OH)$_2$D) (Fig.1-3) (104, 111). Indeed, the main source of vitamin D3 is ultraviolet B radiation with a wave length of 290 to 315 nm which penetrates dermal tissue cells (112). Parker-Autry (2012) and reported that vitamin D can be synthesised in the dermis or digested via certain foods and supplements, which can be metabolised by both the liver and kidney (113). Ultraviolet B waves are maximally absorbed between 11:00am and 2:00pm (114, 115) through the epidermis where 7-dehydrocholesterol is activated to produce previtamin D3 (108, 110, 114). This is then transported to the liver through the bloodstream and hydroxylated to produce 25-dihydroxyvitamin D (25(OH)$_2$D), after which it is hydroxylated in the kidneys to produce 1,25-dihydroxyvitamin D (1,25(OH)$_2$D), a process regulated by PTH. Biologically, 1,25(OH)$_2$D is the key to sustaining normal serum concentrations of calcium and phosphorus ions inside the human body, which are crucial for normal bone mineralisation. Intestinal calcium and phosphorus absorption is increased as a result of binding of 25(OH)$_2$D to vitamin D receptors (VDR) in the intestine (108).

Hamilton (2010) assumes that calcium absorption, which occurs in the small intestine, may be regulated by vitamin D (116). In the case of vitamin D
deficiency, intestinal calcium absorption is reduced, which results in a decline in the serum concentration of calcium ions. This decline can be identified in the parathyroid glands through calcium sensors, and a reduction of calcium ions leads to increased production of PTH, which acts on the kidneys to activate increased production of 1,25(OH)₂D (110, 116-119) (Fig. 1-3).

![Synthesis and mechanism of action of vitamin D](image)

**Figure 1-3.** Synthesis and mechanism of action of vitamin D (118).

Holick (2007) defined vitamin D deficiency as a disease which occurs when serum 25-hydroxyvitamin D (25(OH)D) concentration is less than 20ng/ml (50nmol/L) (Table 1-2) (108, 118, 120). Vitamin D is known as the “sunshine vitamin” (110, 111). Many people suffer from low serum levels of vitamin D which
has been attributed to insufficient exposure to sunlight (121, 122) as well as a lack of vitamin D in the diet (112). However, the main source of vitamin D is exposure to sunlight and only a small amount (around 30%) of vitamin D can be obtained from the diet (7, 121).

1.5.2 Vitamin D deficiency

Vitamin D deficiency is a global issue which affects the majority of people in the world, both healthy people and those with common diseases, children and the elderly (104, 108). Vitamin D deficiency is also linked strongly with colon cancer, arthritis and cardiovascular problems (104, 108, 116, 118, 123). It can lead to skeletal pain and reduced motor function which limits exercise (124), and therefore directly and indirectly it can be considered as a risk factor for coronary heart disease in diabetic patients (118, 125). Studies have shown that a longer exposure to ultraviolet radiation can improve cardiovascular endurance in healthy individuals (116, 123). Low levels of vitamin D can contribute to abnormalities in the musculoskeletal system (108, 119).

1.5.2.1 Levels of vitamin D deficiency

The level of vitamin D concentration varies between people based on amount of sun exposure, type of skin colour, level of obesity, and whether they suffer from diabetes, liver or kidney diseases (112, 126).
Table 1-2. Serum 25(OH)D cut-offs for vitamin D deficiency.

<table>
<thead>
<tr>
<th>25(OH)D concentration</th>
<th>Vitamin D level</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;75nmol/L (&gt;30ng/ml)</td>
<td>Sufficient</td>
</tr>
<tr>
<td>50-75nmol/L (20-30ng/ml)</td>
<td>Insufficient</td>
</tr>
<tr>
<td>&lt;50nmol/L (&lt;20ng/ml)</td>
<td>Deficient</td>
</tr>
<tr>
<td>&lt;25nmol/L (&lt;10ng/ml)</td>
<td>Severely deficient</td>
</tr>
</tbody>
</table>

Adapted from (125, 127, 128). Serum level of 25(OH)D concentration <10 ng/ml (121).

The majority of patients with T1DM and T2DM are vitamin D deficient (<20 ng/mL or insufficient (20-30 ng/ml) (129, 130). One study found that 86% of patients with T2DM had severe vitamin D deficiency (104, 126).

1.5.2.2 Prevalence of vitamin D deficiency

Vitamin D deficiency has become a global problem affecting people in different countries including those with plenty of sunshine such as those in the Middle East, Australia, Turkey and India, with the prevalence of vitamin D deficiency in these countries ranging from 30% to 50% in children and adults with a serum 25(OH)D level of <20ng/ml (104). A high prevalence of vitamin D deficiency is
found in elderly Saudi people (131). However, adolescent females in Australia and England are commonly affected (116). A study found that more than 84% of African males and females aged over 65 years in Boston had vitamin D deficiency (123, 132). Low serum levels of vitamin D have also common in elderly Europeans (128), particularly elderly women (128). The Netherlands and Italy have a high prevalence of vitamin D deficiency (133) and people who live in China and India are also at high risk of vitamin D deficiency (134). The most common reason for this deficiency is lack of exposure to the sun (116). Indeed, the presence of sunshine does not equate to adequate exposure as many societies will cover themselves up and indeed some regions, such as the Middle East, have a very high proportion of people with vitamin D deficiency ranging from 50% to 97% (105).

1.5.2.3 Risk factors for vitamin D deficiency

Many important variables are considered as risk factors for vitamin D deficiency. These include lack of exposure to the sun during the summer, higher levels of skin pigmentation, a higher BMI, time of the day and seasonal changes (108, 110, 112, 115, 131). In the months with the most sunshine (July, August and September), participants have higher serum vitamin D concentration compared to the winter months (127). However, in the UK where sunshine is minimal all year around, the seasonal effect is not significant (101).
Another important factor that increases the possibility of vitamin D deficiency is advancing age (10, 109, 110, 115, 135). Elderly people are more likely to be vitamin D deficient, particularly as many live indoors most of the time as a result of illness and generally reduced physical activities (136). This leads to a reduction in the ability of the skin to absorb UVB radiation (115, 135). In the elderly, a reduction in skin thickness with ageing, combined with a decrease in the skin levels of 7-dehydrocholesterol, reduces vitamin D synthesis, which at the age of 70 is reduced to 30% of the capacity of a 20 year-old person. Thus, even after exposure to the sun it is possible that older people will not produce the amount of vitamin D required to maintain optimal serum 25(OH)D levels (137). Indeed, ageing people have reduced skin production of vitamin D, despite comparable exposure to sunlight as young subjects (123).

Cannell and Hollis (2008) have also reported that despite the fact that many elderly people live in high-sunshine areas, their serum levels of 25-hydroxivitamin D (serum 25(OH)D) are ≤30 nmol/L, i.e. they have vitamin D deficiency (132, 133). The detrimental effects of vitamin D deficiency are further amplified in the elderly as there is a reduction in vitamin D receptors within skeletal muscle due to muscle atrophy (108). This atrophy can be observed particularly in type II fast-twitch muscle fibres with advancing age (109, 136, 138), which happens as people get older and leads to a reduction in muscle strength and an increase in the frequency of falls (108, 109, 139, 140). Thus, with ageing, falls increase by 10% per decade. By the age of 65, one in three people fall at least once a year, and by the age of 80, between one and two falls a year are reported (141). 20%
to 30% of those who fall have moderate to severe injuries such as fractures (141).

Gender is another important variable which increases the possibility of vitamin D deficiency (109). This particularly affects women who use sun block which prevents penetration of sun radiation into the human skin, thus limiting skin synthesis of vitamin D (110). Moreover, many Muslim women wear traditional clothes which cover most of their body, for example, wearing the hijab or a hat to cover the head and long dresses to cover the arms and legs. Therefore, gender has been identified as a key factor in the development of vitamin D deficiency and the prevalence of vitamin D deficiency is higher in women than men (123, 129).

1.5.2.4 Vitamin D deficiency in relation to parathyroid hormone

There is an inverse correlation between serum level of 25(OH)D, PTH and a direct correlation with BMD (121, 131). Subjects with vitamin D deficiency (serum 25(OH)D ≤15 ng/ml) have a reduction in bone mass, particularly at the hips and wrists joints (142).
1.5.2.5 Vitamin D deficiency in relation to musculoskeletal pain

Vitamin D plays a major role in calcium and phosphate handling and bone mineralisation (110, 112). However, vitamin D can also act as a neurotrophic substance which modulates neuronal growth and neuromuscular function (143). Cannell et al. (2008) reported that South Asian women have a high percentage of chronic musculoskeletal pain due to vitamin D deficiency (132). Lower vitamin D levels are associated with a higher proportion of skeletal pain, which is commonly experienced in patients with osteomalacia in the form of generalised bone pains but also particularly when pressing the cortex over the anterior tibia (142). Vitamin D deficiency is strongly associated with low back pain, particularly in elderly women (121).

Another explanation for why people with vitamin D deficiency report pain is because of a decline in calcium absorption and an increase in PTH as a result of vitamin D deficiency. Insufficient calcium and phosphorus ion concentration in the bone leads to inadequate mineralisation of the collagen matrix of the endosteum (internal layer of the bone) which hydrates and swells, and increased pressure on the innervated periosteum (outer layer of the bone) leads to bone pain (142). Skeletal pain is associated with proximal skeletal muscle weakness and impaired gait (143, 144). Patients with low levels of vitamin D initially suffer from musculoskeletal pain mainly in the proximal joints such as shoulder and pelvic girdles, rib cage, lower back pain and lower extremities (113, 143, 145, 146). This pain reduces lower extremity function which increases the risk of falling and proximal bone fractures in elderly people (140, 147-149). It also affects elderly
women more than men, owing to the difference in the anatomical architecture of women’s bones in comparison to men (121, 142). Vitamin D also has anti-inflammatory properties so deficiency may increase muscle inflammation resulting in pain (150).

1.5.2.6 Vitamin D deficiency in relation to the musculoskeletal system

Muscle biopsies in patients with vitamin D deficiency show atrophy in type II muscle fibres in relation to a reduction in muscle strength, which consequently results in a reduction in skeletal muscle size (112, 148, 149). Weakness in muscle strength can lead to a disturbance in balance and inadequate physical function (119, 120, 151). An impairment in muscle performance can be seen with low serum levels of 25(OH)D (119), especially when it is less than 20 nmol/L (141). Limitation of physical performance includes a reduction in standing up from a sitting position, climbing stairs and walking (119, 146). Increased body sway during walking and impaired balance leads to an increased frequency in falls and fractures among elderly individuals (128, 133, 141, 152). Osteoporosis further predisposes sufferers to fractures of skeletal bone and of course this risk is increased with the risk of falling (116).

Skeletal muscle weakness (myopathy) and atrophy are related to low vitamin D levels (121, 143, 153). Insufficient levels of vitamin D can cause myopathy of the hip flexors, knee flexors and extensors as well as a reduction in speed (119),
difficulties in climbing stairs in particular, a disturbance in dynamic medio-lateral body sway and an increased risk of falls in otherwise healthy elderly people (112, 116, 154) and children (146). Whilst some studies have found no evidence that vitamin D alone can improve muscle strength and function in elderly people (121), others have concluded that increasing 25(OH)D3 levels can improve functional performance, reaction time, balance and receptive response without a change in muscle strength in the lower limbs of elderly people (152). There are studies showing that treatment with vitamin D has a direct impact on skeletal muscle function and size, muscle strength and speed of muscle contraction in healthy subjects (117, 155). It has also been reported that cholecalciferol supplements are effective in increasing lower extremity muscle strength (137). One reason for the differing findings is that the vitamin D replacement regimens were inadequate.

1.5.2.7 PTH, VDR and falls

A high serum level of PTH is related to muscle weakness and appears to be an independent risk factor for falls (153, 156-158). PTH has a direct effect on skeletal muscle, and increasing intracellular calcium concentration (159) may disturb muscle structure and function (158). Skeletal muscle cellular function and maturation can be directly affected by vitamin D metabolites via vitamin D receptors located within skeletal muscle. There is a positive relationship between older age and declining VDR gene expression (160) that is independent of biopsy
location. The reduction of serum vitamin D levels which is common in elderly subjects may cause a reduction in vitamin D receptor activation (128, 160).

1.6 Hypotheses and objectives

Diabetes may impair muscle function, structure and balance during normal walking in patients with diabetes. Furthermore, there are numerous studies indicating that vitamin D deficiency causes muscle weakness, atrophy and disturbance of balance (dynamic medio-lateral sway) and walking in healthy subjects. To the best of the author’s knowledge, no study has been done to investigate the relationship of vitamin D deficiency on muscle strength and structure and normal walking strategy in patients with T2DM and subjects with impaired glucose tolerance. The primary hypothesis of this study is that impairment of skeletal muscle strength and size of the lower extremities, biomechanical alterations in balance (medio-lateral sway) and walking pattern occur in subjects with IGT and patients with T2DM and mild neuropathy and that this is related to vitamin D deficiency, intramuscular fat and severity of neuropathy.
1.6.1 Objectives

1. To assess maximal isometric muscle strength of knee extensors and ankle plantar flexors in relation to vitamin D deficiency, intramuscular fat and severity of neuropathy in subjects with IGT and patients with T2DM.

2. To assess muscle volume of the knee extensors, flexors, ankle plantar flexors and dorsiflexors in relation to vitamin D deficiency, intramuscular fat and severity of neuropathy in subjects with IGT and patients with T2DM.

3. To assess walking and balance alterations (dynamic medio-lateral sway) in relation to vitamin D deficiency and severity of neuropathy in subjects with IGT and patients with T2DM.

All assessments were undertaken in subjects with IGT and patients with T2DM and healthy control subjects.

To apply these techniques in subjects with IGT and patients with T2DM, a study was undertaken in healthy control subjects to evaluate the following:

1. The reliability and repeatability of the assessment of knee extensor and ankle plantar flexor muscle strength using isokinetic dynamometry (Cybex).

2. The reliability and repeatability of the assessment of knee extensor, ankle plantar flexor and dorsiflexor muscle volume quantification using MRI.

3. The reliability and repeatability of the assessment of BMD using a DEXA scanner.
1.7 References


84. Mueller MJ, Minor SD, Sahrmann SA, Schaaf JA, Strube MJ. Differences in the gait characteristics of patients with diabetes and peripheral neuropathy


106. Larsson S, Jones HA, Goransson O, Degerman E, Holm C. Parathyroid hormone induces adipocyte lipolysis via PKA-mediated phosphorylation of


2 CHAPTER II. RESEARCH DESIGN AND METHODS
2.1 Participant enrolment

All participants were informed of the nature of the study and were given approximately one week to consider their participation and to discuss any concerns related to the study. They were asked to provide written informed consent before participating. The study was approved by the National Health Service (NHS) Research Ethics Committee and ethics committees of the University of Manchester and Manchester Metropolitan University. This research adhered to the tenets of the declaration of Helsinki. Each participant was asked about their medical history, and inclusion and exclusion criteria before participation. Patients with T2DM, with and without diabetic peripheral neuropathy (DPN) and subjects with IGT were recruited from the Manchester Diabetes Centre and the Manchester Royal Infirmary, Manchester, UK. Healthy control subjects were recruited from the University of Manchester and Manchester Metropolitan University staff and from healthy relatives of participants.

2.2 Inclusion criteria

1. Aged between 40 and 80 years old.

2. Ability to walk independently without using an assistive device.

3. With or without vitamin D insufficiency or deficiency (25(OH)D <50 or 25nmol/L respectively) for subjects with IGT and patients with T2DM.

5. Subjects with IGT and patients with T2DM diagnosed according to an OGTT (IGT-2-hour post glucose load ≥7.8 and <11.1; patients with T2DM ≥11.1) (see chapter 1 for more details).

6. Participants capable of understanding the procedures and providing written informed consent form.

2.3 Exclusion criteria

1. Severe musculoskeletal, neurological, orthopaedic or surgical problems.

2. Severe foot deformities and current foot ulcer.

3. Foot and/or leg amputations.

4. Currently taking vitamin D supplements.

5. Additional exclusion criteria for MRI scanning included participants with epilepsy, a cardiac pacemaker, metal pins/plates and implants from previous operations, metallic fragments in the eyes, and for females pregnancy or a contraceptive diaphragm in situ (chapters 3, 4, and 5).

2.4 Study subjects

The following subjects relate to chapters 4, 5 and 6:
- 20 patients with type 2 diabetes (15 males and 5 females; 8 with mild peripheral neuropathy and 12 without neuropathy, based on Toronto criteria).
- 20 subjects with impaired glucose tolerance (16 males and 4 females; 10 with and 10 without neuropathy).
- 20 healthy control subjects (13 males and 7 females).

**The following subjects relate to chapter 3 only:**
- 12 randomly selected healthy subjects (8 males and 4 females) for reliability measurements.

All patients with T2DM and IGT underwent assessment of anthropometric, metabolic laboratory tests and neuropathy assessments for both large and small nerve fibres (chapters 4, 5 and 6).

Each participant underwent two clinical assessment sessions. In the first visit, the participant was asked about their medical history, and inclusion and exclusion criteria, and they underwent assessment of anthropometric measures, maximal skeletal muscle strength and muscle size of the lower extremity and BMD. During the second visit, assessment of gait analysis was undertaken at the muscle function laboratory at Manchester Metropolitan University, UK.
2.5 Study design

2.5.1 Assessment of neuropathy (chapters 4, 5 and 6)

The severity of DPN was evaluated using the modified neuropathy disability score (NDS) which is composed of tests for the detection of various stimuli on the plantar surface of the foot and the hallux: pinprick sensation (using Neurotip) (Fig. 2-1), vibration (using 128-Hz tuning fork) (Fig. 2-2), and differences in temperature sensation (using warm and cool rods) (Fig. 2-3) as well as assessment of the presence/absence of the Achilles tendon reflex (using tendon hammer) (Fig. 2-4). The NDS ranged from (0 to 10) and participants were stratified into the following groups: no neuropathy (NDS 0 to 2), mild neuropathy (NDS 3 to 5), moderate neuropathy (NDS 6 to 8) and severe neuropathy (NDS 9 to 10) (1).
Figure 2-1. Pain sensation assessment using pinprick.

Figure 2-2. Vibration sensation assessment using a 128 Hz tuning fork.

Figure 2-3. Temperature sensation assessment using cold and hot metal rods.
Figure 2-4. Achilles tendon reflex assessment.

Electro-diagnostic studies were performed using a Dantec “Keypoint” system (Dantec Dynamics Ltd, Bristol, UK). Peroneal motor nerve conduction velocity (PMNCV), peroneal motor nerve amplitude (PMNA), sural nerve conduction velocity (SNCV) and sural nerve amplitude (SNAP) were assessed by a consultant neurophysiologist (Dr. Andrew Marshall) using surface stimulating and recording electrodes (Fig. 2-5). Skin temperature was kept between 32 and 35ºC using a Dansk Industrial Syndikat temperature regulator.

Participants’ skin was scrubbed using an alcohol swap. Surface electrodes were used for stimulating and recording the stimulus response which travelled along the nerve fibre. NCV was reported in (m/s) and maximum amplitude was quantified for the peroneal and sural motor nerves. The strength of the stimulus
was increased gradually until the maximum response was reached, followed by a 10 to 15% increase of the strength of stimulus above the maximal response to achieve a supra-maximal response. The amplitude of the motor response (M wave) was measured from the baseline to the negative peak and expressed in mV. Motor latency was measured from the point of application of the stimulus to the peak of the negative component of the M wave.

For SNAP, the surface recording electrode was placed posterior to the lateral malleolus to detect the stimulus. The surface stimulating electrode was placed 14cm proximal to the recording electrode on the posterior aspect of the distal leg (Fig. 2-5). For PMNA, the recording electrode was placed 1 to 2cms medial to the lateral malleolus of the ankle joint, while the stimulating electrode was placed 12 to 14cms proximal to the recording electrode, anterior to the anterior edge of the fibula. The ground electrode was positioned in between the recording and stimulating electrodes (2).

DSPN was assessed using the Toronto criteria (3) (more details in chapter 1).
2.5.2 Corneal confocal microscopy (chapters 4, 5 and 6)

All study subjects were scanned with a laser IVCCM [Heidelberg Retinal Tomograph III Rostock Cornea Module (Heidelberg Engineering GmbH, Heidelberg, Germany)]. All images were undertaken using the “section” mode in the Heidelberg Explorer of the HRT III RCM and ~6 high clarity images were analysed from the central sub-basal nerve plexus. Corneal nerve fibre damage was assessed by quantifying the following: corneal nerve fibre density (CNFD), defined as the total number of nerve fibres per mm² (no./mm²); corneal nerve branch density (CNBD), the total number of nerve branches per mm² (no./mm²); corneal nerve fibre length (CNFL), the total length of all nerve fibres per mm² (mm/mm²); and corneal nerve fibre tortuosity (CNFT), the degree of non-linearity
of the nerve fibres. These parameters were measured using semi-automated, purpose-written, proprietary software (CCMetrics®, M. A. Dabbah, Imaging Science Biomedical Engineering, University of Manchester, Manchester, UK).

2.5.3 Intraepidermal nerve fibre density (chapters 4, 5 and 6)

Intraepidermal nerve fibre density (IENFD) was quantified in skin biopsies from the dorsum of the foot. A 3mm punch skin biopsy was taken from the dorsum of the foot, approximately 2cm above the second metatarsal head under local anaesthesia (1% lidocaine) and 50μm frozen sections were cut and immuno-stained using anti-human PGP 9.5 antibody (Abcam, UK). Nerve fibres were demonstrated using SG chromogen (Vector, Burlingame, CA) and examined under the Zeiss AxioImager M2 microscope at 400x magnification. IENFD was expressed as no./mm (4).

2.5.4 Measurement of vitamin D (25(OH)D) concentration

Serum 25(OH)D levels were measured in the clinical laboratory (vitamin D laboratory) at Manchester Royal Infirmary, Manchester, UK. The serum was isolated from the blood and was stored at (20⁰ to 25⁰ C) prior to assay. The assay used was an automated platform assay (ImmunoDiagnostic Systems Ltd, Bolden, Tyne and Wear, UK) and is based on chemiluminescence technology. All samples were exposed to a pre-treatment stage to denature the vitamin D
binding protein, then a special anti-25(OH)D antibody labelled with acridinium was added to the treated samples and neutralized in an assay buffer. After incubation, magnetic particles linked to 25(OH)D were added to the treated samples, and after further incubation a magnet was used to capture the magnetic particles. After washing and adding trigger reagents, the light released from the acridinium label was inversely related to the concentration of 25(OH)D in the original sample (5). A 4-point logistic curve was used to calculate 25(OH)D levels automatically. Vitamin D deficiency (<20ng/ml) and insufficiency (<30ng/ml) levels were defined according to the Institute of Medicine of the National Academies (6-11).

2.5.5 Assessment of muscle strength

2.5.5.1 Isokinetic dynamometer (chapters 3, 4 and 5)

Maximal isometric (static) muscle strength for the knee extensors and ankle plantar flexors of the lower limb was assessed using an isokinetic dynamometer (Cybex Norm, Ronkonkoma, NY) (Fig. 2-6). This dynamometer measures the joint torque (Nm) produced around the knee and ankle joints which reflects the amount of muscle force produced by the major muscle groups acting around the knee (knee extensors) and ankle (ankle plantar flexors) joints and the internal tendon moment arm lengths at these joints. The term “muscle strength” will be used in this thesis to indicate the quantified joint torque measured around these joints.
Maximal isometric muscle contraction of the knee extensors and ankle plantar flexors was performed at three different joint angles on the right leg for standardising purposes for all participants. Participants were informed of the nature of the test and what they would feel, how to perform the test and the time interval between and within each set of contractions. Before undertaking the measurements, the full range of joint motion for both knee and ankle joints was assessed and this joint range set into the dynamometer with mechanical safety stops placed just within the extremes of the range of motion (Fig. 2-7).
2.5.5.1.1 **Assessment of knee extensors muscle strength**

*(chapters 3, 4 and 5)*

The maximal joint torque produced by the knee extensors was measured during an isometric maximum voluntary contraction. Participants were seated and secured on the chair of the dynamometer with their knees flexed at 90° and the hip angle at 85° (0° = supine position) whilst their trunk, pelvis and shoulders were stabilized using the dynamometer’s straps. Subjects were asked to place their hands across their chest during all contractions to prevent any extraneous movement (Fig. 2-8). The resistance arm of the dynamometer was attached at the distal part of the tibia using a strap, approximately two inches proximal to the lateral malleolus. The anatomical axis of rotation of the knee joint was palpated and marked using a removable red marker and visually aligned with the axis of rotation of the dynamometer. The participants were instructed and strongly
encouraged to perform a maximal isometric knee extension by pushing as hard as possible against a fixed resistance arm and maintaining this contraction for around 3 to 4 seconds until a verbal command was given to stop and relax. A torque-time curve was presented on a computer screen in front of the participants to encourage maximum effort. Participants were asked to perform three maximal voluntary isometric (static) contractions for the knee extensors at 3 knee joint angles which were assessed in a randomised order: 85º, 70º and 55º, with a two minute rest interval between contractions to prevent muscle fatigue. The highest torque from the three contractions performed at each joint angle was recorded. The mean knee extensor muscle torque across the three different angles was calculated for each participant for further analysis. Initially, the participants were asked to perform 3 to 4 sub-maximal isometric knee extension contractions against the resistance arm as part of a standardized warm-up, which also assisted the participants to be familiar with each test prior to taking measurements.
2.5.5.1.2  Assessment of ankle plantar flexor muscle strength

Maximum isometric (static) muscle contraction of the ankle plantar flexors was assessed using the isokinetic dynamometer as described above. To test ankle plantar flexor muscle strength, participants were asked to lie down in the prone position on the dynamometer with the knee in full extension and the ankle secured in a neutral position (right angle between foot and tibia) by using straps on the footplate of the dynamometer (Fig. 2-9). The ankle joint axis of rotation was aligned with the centre of rotation on the dynamometer (Fig. 2-7). Participants were asked to develop a maximum isometric voluntary contraction of the plantar flexors by trying to plantar flex their right ankle (to push down away from their bodies) as hard as they could against a fixed resistance arm. They
were also asked to hold and keep their maximum contraction for 3 to 4 seconds before relaxing. Maximum voluntary isometric plantar flexor joint torque was assessed at three different joint angles performed in a random order (0º neutral ankle, -5º dorsiflexion and -10º dorsiflexion) and again three maximal isometric contractions were performed and the highest torque was recorded. The mean ankle plantar flexor muscle torque across the three different angles was calculated for each participant for further analysis. Generally, each maximum isometric contraction for both the knee and ankle was held for around 3 to 4 seconds, with a 60 second rest interval between contractions within each angle and 2 minutes between contractions at different angles. The total time for assessment of knee and ankle muscle strength, including the preparation of the subject and dynamometer, was around one hour for each participant.

Figure 2-9. Participant position during assessment of maximal isometric ankle plantar flexor muscle strength.
2.5.6 Assessment of muscle volume (chapters 3, 4 and 5)

2.5.6.1 Magnetic resonance imaging scanner

A 0.25 Tesla MRI peripheral scanner (G-Scan, Esaote, Italy) with coils designed to image different parts of the body (Fig. 2-10) was used to scan the whole length of the lower limb (more details about MRI scanner characteristics are explained in chapters 3, 4 and 5 of the thesis).

[Image of MRI scanner]

Figure 2-10. The 0.25 Tesla peripheral MRI scanner.

2.5.6.1.1 MRI screening questionnaire

There are a number of contra-indications to entering the controlled scanning area of the MRI. Participants were asked about these contraindications and, if any of the exclusion criteria was present, they were not permitted to enter the scanning area and undergo a scan. A screening questionnaire was given to the
participants prior to the testing session. Exclusion criteria for MRI scanning were checked in advance of the MRI.

### 2.5.6.1.2 Participant’s preparation and scanning

Prior to performing upper and lower scans of the lower limb, 5 oil-filled capsules were fixed firmly using adhesive tape starting distally along the shaft of the tibia of the participant’s leg from the lateral malleolus of the ankle with a 10cm gap between each one. Another 4 capsules were fixed along the lateral side of the thigh parallel to the femur with the same distance between each capsule for the upper leg. These capsules represented markers to distinguish between each package of scans during the test and to prevent duplicate reading during the image analysis (Fig. 2-11 A&B) and (Fig. 2-12).
Figure 2-11. Markers for each scanned section along the lower leg (A) and upper leg (B) of a participant.

Figure 2-12. This figure shows the capsule marker on the MRI image.
The participant was asked to lie supine and relax on the MRI scanner bed. Their whole leg was scanned with a fully-extended knee supported with a comfortable pad under the knee joint while the ankle joint was in a neutral position and supported and secured with an appropriate cushion.

Subjects were instructed to keep calm and relax during the test and avoid moving the leg in order to obtain a clear image. Specific coils were used for the lower limb. The examiner asked the participants to put their right leg inside the coil and then checked the capsules’ location carefully to make sure that the required area was scanned completely.

During the test, three scan packages were performed for the lower right leg (lower leg muscles) to obtain images for the plantar flexor and dorsiflexor muscles which act across the ankle joint and another three scan packages were performed for the upper portion of the right leg (thigh) to scan the knee extensor and flexors muscle groups. Therefore, a total of six scanning packages were obtained from the right leg. All scans were performed in order, from the distal to proximal part of the leg. After the first scan was completed (the first third of the lower leg), the participants were asked to move their leg further down for the second scan package of the leg (second part) and again the location of the capsules was checked. At the end of the second scan package, the same process was performed for the most proximal part of the calf muscle (third scan). At the end of the lower leg scan, the knee coil was replaced with a thigh coil and the same procedures were performed to scan the thigh (knee extensor and flexor muscles).
Prior to each scan, a scout (~15 seconds) was carried out to confirm correct limb position in the scanner. The total time for an MRI scan of the whole leg, including preparation and adjustment of the participant’s position for each scan, was approximately one hour.

2.5.6.1.3 Muscle volume calculation

The cross-sectional area (CSA) of each muscle was manually outlined and analysed (Fig. 2-13 and 2-14) from the serial axial plane scans (Fig. 2-15 and 2-16). To make an informed decision on how many CSAs were required to be analysed for each specific muscle in order to provide an accurate muscle volume calculation, three healthy subjects were randomly selected and all of their available CSAs analysed consecutively along the muscle. The muscle volume was calculated using all available slices and then compared against muscle volume calculations using measurements from every second and third slice. Depending on the degree of error in this analysis, some of the leg muscles were analysed using every axial scan slice while other muscles were analysed in every third slice in order to calculate muscle volume (Tables 2-1 & 2-2). Both tables show the percentage error in muscle volume calculation in the ankle plantar and dorsiflexor muscles (Table 2-1) and knee extensor muscles (Table 2-2) across the three healthy subjects. Muscle volume was calculated using every consecutive slice, every 2\textsuperscript{nd} slice, every 3\textsuperscript{rd} slice, every 4\textsuperscript{th} slice and every 5\textsuperscript{th} slice (the number of axial scan slices were dependent on the anatomical length of the
muscle) and the percentage error was calculated for each of the three subjects. The muscle volumes and the percentage error associated with using a certain number of slices are presented in (Tables 2-1 and 2-2). For a given muscle, we deemed a percentage error <5% in comparison to using every data from every slice to be acceptable for the calculation of muscle volume. This pre-analysis procedure determined the optimal number of MRI slices to be analysed for the main studies as an appropriate balance between accuracy and time efficiency.

For the ankle muscles, it was determined that the soleus and dorsiflexor muscles would be analysed using every third slice while the medial (MG) and lateral head of gastrocnemius (LG) muscles would be analysed using every slice of MRI images. For the knee extensor muscles, it was determined that the vastus medialis (VM), vastus intermedius (VI) and vastus lateralis (VL) muscles were to be analysed using every third slice, but the rectus femoris (RF) muscle would be analysed using every slice from the MRI images.

The sum of all CSAs for each muscle was calculated ($\Sigma$CSAcm$^2$), and this was 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$^3$) = ($\Sigma$CSAcm$^2$ * $d$)
Table 2-1. Percentage error in the calculated muscle volume of the ankle muscles by measuring a different number of MRI slices in comparison to using all consecutive MRI slices.

Data shown are the means from three subjects as described above.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Number of axial slices used</th>
<th>Muscle volume (cm$^3$)</th>
<th>% of Error</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ankle plantar flexor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soleus</td>
<td>Each slice</td>
<td>577.0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Each 2$^{nd}$ slice</td>
<td>586.1</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Each 3$^{rd}$ slice</td>
<td>579.6</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>Each 4$^{th}$ slice</td>
<td>583.5</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Each 5$^{th}$ slice</td>
<td>570.1</td>
<td>3.9</td>
</tr>
<tr>
<td>MG</td>
<td>Each slice</td>
<td>166.2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Each 2$^{nd}$ slice</td>
<td>165.5</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>Each 3$^{rd}$ slice</td>
<td>168.6</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td>Each 4$^{th}$ slice</td>
<td>158.0</td>
<td>13.5</td>
</tr>
<tr>
<td>LG</td>
<td>Each slice</td>
<td>85.4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Each 2$^{nd}$ slice</td>
<td>84.9</td>
<td>4.3</td>
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<tr>
<td></td>
<td>Each 3$^{rd}$ slice</td>
<td>81.7</td>
<td>12.4</td>
</tr>
<tr>
<td><strong>Ankle dorsiflexor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Each slice</td>
<td>256.7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Each 2$^{nd}$ slice</td>
<td>254.8</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Each 3$^{rd}$ slice</td>
<td>253.3</td>
<td>4.0</td>
</tr>
<tr>
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<td>Each 4$^{th}$ slice</td>
<td>255.6</td>
<td>3.9</td>
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<tr>
<td></td>
<td>Each 5$^{th}$ slice</td>
<td>255.6</td>
<td>4.1</td>
</tr>
</tbody>
</table>

MG: medial head of gastrocnemius muscle; LG: lateral head of gastrocnemius muscle
Table 2-2. Percentage error in the calculated muscle volume of the knee extensor muscles by measuring a different number of MRI slices in comparison to using all consecutive MRI slices.

Data shown are the means from three subjects as described above.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Axial scan slice</th>
<th>Muscle volume (cm$^3$)</th>
<th>% of Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knee extensors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VM</td>
<td>Each slice</td>
<td>568.1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Each 2$^{nd}$ slice</td>
<td>578.6</td>
<td>1.8</td>
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<tr>
<td></td>
<td>Each 3$^{rd}$ slice</td>
<td>572.8</td>
<td>2.8</td>
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<td></td>
<td>Each 4$^{th}$ slice</td>
<td>581.3</td>
<td>3.6</td>
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<tr>
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<td>Each 5$^{th}$ slice</td>
<td>559.7</td>
<td>2.2</td>
</tr>
<tr>
<td>VI</td>
<td>Each slice</td>
<td>518.4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Each 2$^{nd}$ slice</td>
<td>507.3</td>
<td>2.0</td>
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<td>Each 3$^{rd}$ slice</td>
<td>501.1</td>
<td>3.3</td>
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<td>Each 4$^{th}$ slice</td>
<td>493.9</td>
<td>5.2</td>
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<td>Each 5$^{th}$ slice</td>
<td>510.5</td>
<td>8.0</td>
</tr>
<tr>
<td>VL</td>
<td>Each slice</td>
<td>468.2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Each 2$^{nd}$ slice</td>
<td>482.9</td>
<td>3.1</td>
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<td></td>
<td>Each 3$^{rd}$ slice</td>
<td>461.4</td>
<td>1.4</td>
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<td></td>
<td>Each 4$^{th}$ slice</td>
<td>456.3</td>
<td>2.5</td>
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<tr>
<td></td>
<td>Each 5$^{th}$ slice</td>
<td>466.4</td>
<td>1.5</td>
</tr>
<tr>
<td>RF</td>
<td>Each slice</td>
<td>132.9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Each 2$^{nd}$ slice</td>
<td>126.6</td>
<td>4.8</td>
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<tr>
<td></td>
<td>Each 3$^{rd}$ slice</td>
<td>133.0</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>Each 4$^{th}$ slice</td>
<td>122.7</td>
<td>15.8</td>
</tr>
</tbody>
</table>

VM: vastus medialis; VI: vastus intermedius; VL: vastus lateralis; RF: rectus femoris
Figure 2-13 A&B. Outlined and analysed CSAs of the knee extensor muscles (VM, VI, VL, RF) in a healthy control subject (A) and a patient with T2DM (B).

Figure 2-14 A&B. Outlined and analysed CSAs of the ankle plantar flexors (soleus, MG and LG) and dorsiflexor (DF) muscles in a healthy control subject (A) and a patient with T2DM (B).
Serial CSAs of the knee extensors (VM, VI, VL and RF) and knee flexors (semimembranosus, biceps femoris and semitendinosus) (Fig. 2-15); ankle plantar flexors (soleus, MG and LG); and ankle dorsiflexors (tibialis anterior, extensor digitorum longus and extensor hallucis longus) (Fig. 2-16) were manually analysed using digitizing software (OsiriX, Pixmeo, Geneva, Switzerland).

Figure 2-15. Serial axial plane images of the knee extensor and flexor muscles in order to illustrate the concept of calculating muscle volume of the proximal muscles of the lower limb.
2.5.7 Assessment of intramuscular fat (chapters 4 and 5)

2.5.7.1 Intramuscular non-contractile tissue

The presence of different tissues within an MRI image such as fat or connective tissue is reflected by their MRI signal intensity. A low signal intensity value indicates the presence of connective tissue whereas very high signal intensity indicates the presence of fat tissue (Fig. 2-17). The frequency distribution of the signal intensity was measured from outlined CSAs of muscles studied using OsiriX software. The signal intensity of the muscle area was quantified and recorded for each of the muscles studied by measuring the frequency distribution of the signal intensity in the outlined CSA of each single muscle (Fig. 2-18).
Three different points along the participant’s upper and lower legs were chosen according to the origin and insertion of the muscle, and the signal intensity of each muscle area was calculated and recorded. The sum of the three signal intensity values from each muscle was used for further analysis.

Figure 2-17. An illustration of increased intramuscular fat of the lower limb in a participant with IGT and another participant with T2DM compared to a control (C) subject.
Figure 2-18. An example of the frequency spectrum of signal intensities of the CSA of the ankle plantar flexor muscle. The arrow indicates the highest frequency of the signal intensity measured within the CSA of this muscle.

2.5.8 Assessment of bone mineral density (chapter 3)

2.5.8.1 Dual-energy x-ray absorptiometry

The BMD of the whole body was measured using a DEXA scanner (Fig. 2-19). Each participant was asked to lie supine with both hands flat besides their body on the DEXA bed and to remain calm and relaxed without movement during the scan (~15 minutes) in order to obtain the whole body bone density.
2.5.9 Assessment of walking and dynamic sway

2.5.9.1 Gait analysis

Participants were invited to the gait laboratory at Manchester Metropolitan University to assess their gait during level walking. Participants walked at their normal self-selected speed over a 10m walkway, stepping onto two of the three ground-embedded force platforms (Kistler, Winterthur, Switzerland) with a sampling rate of 1000Hz. The movement of markers placed on specific anatomical landmarks of the participant’s body; head, trunk, upper and lower extremities (Fig. 2-22) was assessed using a 10-camera motion-capture system (Vicon, Oxford, UK) sampling at 100Hz (Fig. 2-20) which was fixed around the assessment area to capture the participant’s locomotion. These cameras were calibrated with a specific instrument (Fig. 2-21) before the walking trials of participants were captured.
Figure 2-20. A screen capture from the Vicon software (Vicon, Oxford, UK).

Figure 2-21. The Vicon calibration “wand” used for static and dynamic calibration (Vicon, Oxford, UK).

Participants were informed about the nature of the assessments and asked to take off metals such as gold, accessories, wristwatch etc. in order to avoid
reflections from sources other than the reflective markers. Using a full-body modified Helen-Hayes marker set, 52 plastic reflective markers were fixed firmly using adhesive tape to stabilise them during walking and to prevent any missing data. These markers were placed onto specific anatomical landmarks of the body according to the kinematic model used. Participants were given standardised shoes (MedSurg; Darco, Raisting, Germany) to standardise footwear between participants and also to make sure that patients with diabetes walked safely with appropriate footwear. A tight-fitting t-shirt and shorts were given to participants to minimise marker movement when placed onto clothing (Fig. 2-22).

Figure 2-22. A full-body modified Helen-Hayes marker set placed and fixed onto anatomical landmarks over the body (head, trunk, upper and lower extremities) to enable detection of participant’s locomotion using the motion capture system.
Participants were informed about the test procedures by the examiner both verbally and in practice. After applying the reflective markers onto the participant's body, the participant was asked to stand with their feet apart on the force platforms whilst their arms were in abduction with the person looking straight ahead (Fig. 2-23). Calibration capture was performed to ensure all markers were present and could be detected in the software screen and could then be subsequently labelled (Fig. 2-23). These markers were labelled for each walking trial after each walking assessment had been completed for further analysis (Fig. 2-24).

Figure 2-23. Participant’s calibration image using the Vicon software (Vicon, Oxford, UK) to ensure the correct positioning of the modified Helen-Hayes marker set.
Figure 2-24. Labelling of the full-body modified Helen-Hayes marker set using visual 3D version 5 (Vicon, Oxford, UK).

Three embedded force platforms (Kistler, Winterthur, Switzerland) were used to analyse kinetics at 1000Hz during normal walking (walking on the ground at normal speed). Force platforms were positioned midway along the walkway and embedded into the laboratory floor (Fig. 2-25). After subject calibration, the participant was asked to start walking at their normal self-selected speed from a specific starting point from the beginning to the end of the walkway (10 metres) and then to turn and walk back again to the starting point. Kinetic data were collected simultaneously with the motion analysis data and were taken from trials with a “clean” foot strike onto the force platform (Fig. 2-25). The starting position of walking was shifted slightly forwards or backwards in subsequent walking trials to ensure the best starting point for each subject to achieve a clean foot strike on
the platforms without the participants “targeting” the force platforms. The participant was instructed to repeat these walking trials until four full foot strikes for each leg were achieved. In this study, the aim was to have full foot contact on the force platform (for both right and left) in at least four trials for further analysis. The mean data from four walking trials containing two force platform strikes with the left and two with the right foot were selected for further analysis.

Figure 2-25. Image from the Vicon software showing a subject stepping onto the ground-embedded force platform (Kistler, Winterthur, Switzerland) from which ground reaction forces (kinetic data) were measured.

2.5.9.2 Gait parameters

Joint moments measured during gait reflect the output from the major muscle group acting around that joint to produce motion. The joint moment measured in Nm was normalised to body mass (Nm/kg). Ankle and knee joint moments during
gait have been termed ankle and knee “joint strength” in this thesis to facilitate clinical understanding. Ankle and knee joint strength and power (peak values) during walking (measured by combining the force and motion data) and temporal-spatial parameters (walking speed, stride width and step length, stance time and cycle time) were quantified for each participant using Visual 3D software (C-motion Inc., MD, USA). Peak ankle and knee joint strength and power during walking were calculated for each participant for both right and left legs for each of the trials, taking into consideration each peak value of the joint strength, and power was normalised to body mass. The mean value for all parameters from the four trials was calculated taking into account data from both the left and right legs, with the strength and power values normalised to the participant’s body mass.

2.5.9.3 Dynamic sway during walking

To quantify balance impairment during walking, a variable that is termed “dynamic sway” was calculated. Dynamic sway is a term which is used to describe the separation distance between the body CoM and the CoP during walking. Dynamic sway can be evaluated in two planes; a sagittal (anterior-posterior direction) and a frontal (medio-lateral direction) plane. Higher separation between the CoM and CoP (cm) contributes greater dynamic sway in both directions (Fig. 2-26). The maximum range and mean dynamic sway (in medio-lateral and anterior-posterior planes) was measured during walking.
Figure 2-26. Dynamic sway. Dynamic sway (S) is defined as the separation of the body CoM (grey circle) and the CoP (grey triangle). Dynamic sway is shown in the sagittal (left image) and frontal (right image) planes, illustrating the separation in the anterior-posterior direction and medial-lateral directions respectively. Also highlighted in the sagittal plane image is the resultant ground reaction force vector (grey arrow) and the ankle joint centre position (black cross): joint moments (strength) were calculated based on the relative position and magnitude of the ground reaction force from the joint centre as well as taking into account the mass and acceleration of the relevant body segments.
When the CoM is located in the same vertical line to the CoP (sum to zero), the body becomes more stable in a static position. Increased separation between CoM and CoP causes unsteadiness during walking. The whole body CoM was calculated from the tracked marker data (motion analysis) using the kinematic model. Dempster’s segment parameter model (12) was used to calculate mass distribution for each body segment which allowed the overall CoM to be calculated. The CoP under the foot was calculated using the resultant ground reaction force measurements from the force platforms, and was calculated as a weighted average when the participant’s feet were in contact with two separate force platforms.

Before undertaking any of the above assessments, a pilot study was undertaken in twelve healthy control subjects to evaluate the intra-observer repeatability, agreement and symmetry for the assessment of:

- Knee extensor and ankle plantar flexor muscle strength using the isokinetic dynamometer (Cybex).
- Knee extensor, flexor, ankle plantar flexor and dorsiflexor muscle volume using MRI.
- BMD using DEXA.

### 2.6 Statistical analysis for the current thesis

Data analysis was performed using SPSS statistical software for Windows (version 22) (Chicago, IL, USA). For intra-observer repeatability, intra-class
correlation coefficient (ICC) was used to calculate the agreements of measurements between and within two occasions. The ICC was considered to indicate excellent agreement if it was 0.8 to 1.0, very good if 0.6 to 0.79 and poor if less than 0.51. A 95% confidence interval (CI) (values <0.6 indicate poor reliability) was calculated. A typical error was calculated using the equation: SDdiff / \sqrt{2}. A Bland-Altman plot (the mean difference between the test sessions) was also used to analyse the agreement between measurements of muscle CSAs of the lower limb on two occasions for the same image by the same observer.

Data were analysed using descriptive statistics and presented as mean ± standard deviation (SD). Anthropometric, metabolic, vitamin D and neuropathy assessments; maximal muscle strength (knee extensors and ankle plantar flexors); and muscle volume for knee extensors, flexors, ankle dorsiflexors and plantar flexors, intramuscular fat tissue, total body BMD and walking and balance analysis were analysed and described as mean ± SD, unless otherwise stated.

An independent samples Student’s t-test was used to test the differences between the two study groups in the measured variables (metabolic variables, severity of neuropathy, 25(OH)D, muscle strength, size, intramuscular fat, BMD and walking and balance variables) and also differences in the measured variables between subgroups with and without neuropathy and with lower and higher values of vitamin D (25(OH)D <25nmol/L vs >25nmol/L). Pearson’s Chi-square (χ^2) test of independence and a Fisher’s Exact test were used to evaluate
the association between the categorical variables such as differences in gender and ethnicity between groups.

A one-way analysis of variance (ANOVA) with post-hoc Bonferroni was used to test the differences between the three study groups (T2DM, IGT and control) for the above measured variables.

A Pearson’s correlation coefficient was performed to test the relationship between measured variables and other parameters between study groups. The significance level in all measured variables was P<0.05.
2.7 References


3 CHAPTER III. REPEATABILITY OF MAXIMAL ISOMETRIC MUSCLE STRENGTH, LOWER LIMB MUSCLE VOLUME AND BONE MINERAL DENSITY

Author’s contribution: Monirah Almurdhi contributed to the study design, undertook the repeatability measurements of muscle strength, muscle volume and bone mineral density. She performed statistical analysis and wrote the manuscript, which constitutes the basis of this chapter.

Monirah Almurdhi, Neil Reeves, Frank Bowling, Andrew Boulton, Maria Jezierska and Rayaz A. Malik
3.1 Abstract

**Aim:** To establish the intra-observer repeatability and symmetry of isometric muscle contraction, muscle volume and skeletal BMD in healthy subjects.

**Methods:** Twelve healthy control subjects underwent assessment of the following: 1. Isometric muscle contraction of the knee extensors at 55°, 70° and 85° of knee flexion and ankle plantar flexors at 0, -5° and -10° of dorsiflexion, using the isokinetic dynamometer; 2. Muscle volume using MRI; and 3. BMD using DEXA on two occasions within 7 days. The ICC was used to assess the agreement between measurements.

**Results:** The ICC showed that there was excellent reliability for measures of strength at all three angles at the knee (0.95-0.98) and ankle joints (0.96–0.98) (P= 0.000). The ICC for MRI images was (0.96-0.99) and 0.99 for the BMD measurements (P= 0.000). Females had weaker knee extensor muscles than males (P= 0.033, 0.042 and 0.042) whereas there was no significant difference in muscle strength for the ankle plantar flexors between both genders (P= 0.08, 0.14 and 0.06). Detailed analysis of the MRI images showed that muscle volume was significantly lower in females compared to males (P= 0.01) except for the RF (P= 0.49). BMD assessment showed there were no significant variations between males and females.

**Conclusion:** This study has demonstrated excellent repeatability for the measurements of maximal isometric muscle contraction of knee extensors and
ankle plantar flexors, lower limb muscle volume and BMD. There were significant differences in muscle strength and volume between males and females.

Keywords

Maximal isometric muscle contraction, muscle volume, bone mineral density, intra-class correlation coefficient.
3.2 Introduction

DPN is the most common and highly prevalent complication of diabetes (1). Patients aged greater than 60 are more likely to have DN (2). Sensory and motor deficits can affect their daily physical activities such as walking and climbing stairs which in turn affect their quality of life. Sensory deficits can be present in subjects with IGT and at diagnosis of T2DM. These can affect the lower limbs and can progress to include motor problems (3). Motor dysfunction typically starts later and affects the ankle joint predominantly although it can progress proximally to affect knee and thigh muscles, with quite marked muscle atrophy. There is a significant association between MNCV and muscle atrophy (4). Thus, reduced NCV, muscle weakness, muscle atrophy, tendon stiffness and a limited range of motion are associated with reduced skeletal muscle flexibility in patients with diabetes (5). Distal knee (flexors and extensors) and ankle (dorsiflexors and plantar flexors) muscles have a reduced muscle strength and size which impacts on the quality of life in patients with diabetes mellitus (1, 6). Motor impairment is related to the severity of DN (6). An isokinetic dynamometer is commonly used for the assessment of mild to moderate muscle weakness in diabetic patients. Both T1DM and T2DM patients suffer from weakness of distal muscle strength particularly at the knee and ankle joints. This can result in decreased muscle function and altered functional daily activity (6). To assess and compare skeletal muscle strength and performance between healthy subjects and patients, it is essential to make sure that the measurements undertaken are reliable for the knee and ankle joints across the range of joint angles to be tested (7). Maximal
Voluntary muscle contractions (MVC) of the knee and ankle joints can be measured using an isokinetic dynamometer (Cybex) (8). Testing maximal isometric contraction of the knee extensors (quadriceps femoris) has been shown to be more reliable than testing of the knee flexors; isometric contractions for the knee extensors and flexors are also more reliable in comparison to testing isokinetic contractions at different speeds (7). Repeatability assessment of isometric muscle strength using the isokinetic dynamometer for both knee extensors and flexors has been shown to be highly reliable, safe and easy, with an excellent ICC (0.88-0.92) for both knee extension and flexion (9, 10).

MRI is a diagnostic instrument which provides a non-invasive measurement with no ionizing radiation that gives high quality, detailed information about the architecture of the soft tissues, such as skeletal muscles. This can be used to calculate the CSA and volume of each single muscle (11-14). This tool has commonly been used in healthy subjects and patients with muscular disorders (11). The reliability of repeated measurements of the CSA of spinal muscles is very high (11); the assessment of ankle dorsiflexor muscles has proved very reliable in healthy subjects (15) and in the assessment of quadriceps muscle volume in healthy young and elderly subjects (ICC>0.99) (16). It can be used to diagnose muscle atrophy in patients with diabetes (6) and DN (5).

Vitamin D deficiency is linked strongly with colon cancer, arthritis and cardiovascular problems (17-19), and is a risk factor for coronary heart disease in diabetic patients (20). Low levels of vitamin D can affect both healthy people and people with diabetes mellitus and can contribute to abnormalities in the bones.
and muscles (19). Low levels of vitamin D in its most florid form manifests as osteomalacia and bone deformity as well as proximal myopathy due to a disturbance in calcium handling with altered muscle metabolism (19, 21). Vitamin D deficiency may affect BMD; DEXA can be used to measure bone density and body composition. Body composition measurements include total body fat, lean tissue and bone mineral content. A DEXA scan exposes the human body to very small amounts of radiation during an examination (≤15μSv) (22). The defects in the musculoskeletal system which affect muscles and bones can occur in apparently healthy people with vitamin D deficiency (23) and may be even more pronounced in patients with diabetes mellitus (24). Therefore, before we can define an abnormality in motor function in patients with T2DM and IGT, measurement of the reliability and repeatability of the tests for muscle strength, muscle size and BMD are essential.

Thus, a study was undertaken to establish the intra-observer repeatability, agreement and symmetry of isometric muscle contraction of knee extensor and ankle plantar flexor muscles using isokinetic dynamometer (Cybex), MRI to establish muscle volume, and DEXA for BMD measurement.

### 3.3 Methods

#### 3.3.1 Study subjects

Twelve randomly selected healthy subjects (8 males and 4 females), (5 European and 7 Asian) with a mean ± SD age 31.8±7.2 years, height 1.71±0.12
m, body mass 76.7±13.7 kg and body mass index (BMI) 26.1±3.4 kg/m² were invited to take part in this study. Participants were recruited from the local area around the University of Manchester and Manchester Metropolitan University, and their assessments were conducted in the Institute for Biomedical Research into Human Movement and Health (IRM) muscle function laboratory at Manchester Metropolitan University. They did not have diabetes mellitus or vitamin D deficiency (25(OH)D<25nmo/L). Subjects with severe musculoskeletal problems, orthopaedic or surgical problems, those who were obese and pregnant women were excluded from the study. All participants were informed about the nature of the study and were asked to provide written informed consent before participating. The study was approved by the local research ethics committees at the University of Manchester and Manchester Metropolitan University.

3.3.2 Experiment design

Each participant was tested on two occasions, separated by a 1-week interval for reliability measurements.

3.3.3 Anthropometric measurements

Participants underwent assessment of height, body mass, maximal skeletal muscle strength, muscle size and BMD. All measurements were tested on 2 occasions, except height and weight measurements which were measured at the first visit only. The first measurements provided a baseline and after a week
these assessments were repeated by the same examiner on the same participants and in the same situations for reliability measurements.

3.3.4 Isokinetic dynamometer (Cybex)

Maximal isometric muscle strength for knee extensors and ankle plantar flexors was assessed using an isokinetic dynamometer (Cybex Norm, Ronkonkoma, NY) at three different angles for both the knee (55°, 70° and 85° of flexion) and the ankle (0, -5° and -10° of dorsiflexion) joints.

3.3.5 Magnetic Resonance Imaging

Muscle volume of the knee extensor and ankle plantar flexor muscles was analysed using a 0.25 Tesla peripheral MRI scanner. Scan slices were taken using a Gradient Echo scan with the following parameters: field of view= 200x200; matrix= 256x192; slice thickness= 10mm, spaces between slices (interslice gap)= 1mm; time to echo= 16ms; time to repetition= 685ms and flip angle was 90 degrees. Each scan lasted 3 to 5 minutes. Individual muscle CSAs were calculated for the quadriceps (VM, VI, VL and RF), ankle plantar flexor muscles (soleus, MG and LG) and ankle dorsiflexor muscles (tibialis anterior and extensor digitorum and hallucis longus) (Fig. 3-1). Muscle volume for each muscle was calculated using Osirix software. The sum of all recorded CSAs for each muscle was calculated ($\sum \text{CSA}_\text{cm}^2$) and multiplied by the distance between each muscle
section (d) in order to derive the actual muscle volume (cm³), using this equation 
\( \sum_{i} \text{CSA}_i \cdot d \).

Figure 3-1. Measurements of CSAs in each single muscle of the proximal muscles (A) and distal muscles (B) in the lower limb in healthy subject. VM= vastus medialis; VI= vastus intermedius; VL= vastus lateralis; RF= rectus femoris; MG= medial head of gastrocnemius; LG= lateral head of gastrocnemius; DF= dorsiflexor muscles.

**3.3.6 Dual-energy x-ray absorptiometry**

BMD of the whole body was measured using a DEXA. A very small dose of radiation was involved for the assessment (0.4 µGy).
3.3.7 Statistical analysis

Data analysis were performed using SPSS statistical software for Windows (version 20). Data was analysed using descriptive statistics and presented as mean ± SD. Age, height, body mass, maximal muscle strength and volume and total body BMD were measured.

The ICC was used to calculate the agreement of measurements between and within occasions. The ICC was considered to be in excellent agreement if it was 0.8 to 1.0, very good if 0.6 to 0.79, and poor if less than 0.51. A 95% CI (values <0.6 indicate poor reliability) was calculated. The typical error was calculated using this equation: $SD_{\text{diff}}/\sqrt{2}$. A Bland-Altman plot (the mean difference between the test sessions) was also used to analyse the agreement between measurements of CSAs of leg muscles (knee extensors and ankle plantar flexors) on two occasions for the same image by the same observer. Two independent sample tests (Mann-Whitney U test) were used to assess differences between males and females.

3.4 Results

3.4.1 Maximal isometric muscle contraction

Maximal voluntary contraction of the knee extensors was conducted in the right knee joint when it was flexed at three different angles (55°, 70° and 85° degrees) against maximum resistance produced by the resistance arm. These degrees are considered as the starting position of the knee joint to perform an extension
movement. Table 3-1 shows that maximal isometric strength of the knee extensor muscles was significantly lower at 55º (196.8±76.5 Nm) compared to 70º (231.2±96.8 Nm) and 85º (227.7±100.4 Nm). The ICC showed excellent reliability between visit 1 and visit 2 for all three angles at the knee joint. There was higher agreement at 85º (0.983; 95% CI 0.940/0.995) than 55º (0.956; 95% CI 0.823/0.988) and 70º (0.976; 95% CI 0.917/0.993) (P= 0.000) (Table 3-1). Maximal isometric muscle contraction of the plantar flexor muscles was significantly higher at -10º in dorsiflexion (121.7±36.9 Nm) compared to -5º (112.7±35.4 Nm) and 0 (neutral position of the ankle joint) (102.8±31.9 Nm) (Table 3-1). Repeatability measurements showed excellent reliability with ICC at 0, -5º and -10º, 0.980, 0.967 and 0.977 respectively. Typical error calculations were lower at the ankle joint compared with the knee joint at all three degrees (6.8, 9.7, 8.7) and (19.8, 22.4, 20.0) respectively (Table 3-1).
### Table 3-1. Intra-observer repeatability of MVC at knee and ankle joints at different angles on two separate occasions.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MVC v1</th>
<th>MVC v2</th>
<th>Typical Error</th>
<th>ICC</th>
<th>95%CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MVC_Knee 55º(Nm)</strong></td>
<td>196.8±76.5</td>
<td>180.1±76.4</td>
<td>19.8</td>
<td>0.956</td>
<td>0.823/0.988</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>MVC_Knee 70º(Nm)</strong></td>
<td>231.2±96.8</td>
<td>227.1±103.7</td>
<td>22.4</td>
<td>0.976</td>
<td>0.917/0.993</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>MVC_Knee 85º(Nm)</strong></td>
<td>227.7±100.4</td>
<td>226.7±107.9</td>
<td>20.0</td>
<td>0.983</td>
<td>0.940/0.995</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>MVC_ankle 0º (Nm)</strong></td>
<td>102.8±31.8</td>
<td>103.9±34.5</td>
<td>6.8</td>
<td>0.980</td>
<td>0.929/0.994</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>MVC_ankle (-5º) (Nm)</strong></td>
<td>112.7±35.4</td>
<td>113.5±38.7</td>
<td>9.7</td>
<td>0.967</td>
<td>0.885/0.990</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>MVC_ankle (-10º) (Nm)</strong></td>
<td>121.7±36.9</td>
<td>121.3±43.3</td>
<td>8.7</td>
<td>0.977</td>
<td>0.921/0.993</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD, (MVC;Nm) at the knee joint at different angles (55, 70 and 85 degrees in flexion) and ankle joints (0, -5 and -10 degrees in dorsiflexion), v1= visit 1, v2= visit 2. ICC= 95% CI with lower/upper bound. Typical error= S.D $_{diff}$/√2.

There were no significant differences between 70º and 85º (P= 0.66) (P >0.05) and 55º and 85º (P= 0.099) but there was a significant difference between 55º & 70º (P= 0.01) (P <0.05) at the knee joint. Muscle strength at the knee at 70º (231.2±96.8 Nm) was significantly higher than at 55º (196.8±76.5 Nm).
Significantly higher muscle strength was found at -10° (121.7±36.9 Nm) compared -5° (112.7±35.4 Nm) and 0 (102.8±31.8 Nm) (Table 3-2).

Table 3-2. Comparison of MVC between each knee and ankle angle.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MVC</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVC_knee 55° &amp; knee 70°</td>
<td>(196.8±76.5) &amp; (231.2±96.8)</td>
<td>0.01</td>
</tr>
<tr>
<td>MVC_knee 55° &amp; knee 85°</td>
<td>(196.8±76.5) &amp; (227.7±100.4)</td>
<td>0.099</td>
</tr>
<tr>
<td>MVC_knee 70° &amp; knee 85°</td>
<td>(231.2±96.8) &amp; (227.7±100.4)</td>
<td>0.66</td>
</tr>
<tr>
<td>MVC_ankle 0 &amp; -5°</td>
<td>(102.8±31.8) &amp; (112.7±35.4)</td>
<td>0.008</td>
</tr>
<tr>
<td>MVC_ankle 0 &amp; -10°</td>
<td>(102.8±31.8) &amp; (121.7±36.9)</td>
<td>0.003</td>
</tr>
<tr>
<td>MVC_ankle -5° &amp; -10°</td>
<td>(112.7±35.4) &amp; (121.7±36.9)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Results are presented as mean ± SD. P values <0.05. Non-parametric test for 2 related samples were used.

3.4.2 MRI and DEXA analysis

Of the ankle plantar flexor muscles, the soleus had the largest area and the LG had the smallest area which was approximately half of the MG. For the quadriceps group, the VM, VI and VL were comparable in size. The repeatability of MRI images showed excellent reliability with an ICC >0.90. A typical error was higher for the soleus muscle (10.6) compared with the other calf muscles; MG, LG and dorsiflexors (4.8, 3.3, and 6.9 respectively) and it was also higher for VI (18.3) compared with the VM (10.3), VL (7.4) and RF (5.5). The CSAs in the calf muscles (Table 3-4) compared with the calculated muscle volume of the same muscles (Table 3-3) did not differ significantly. ICC was >0.90 for all images with a P value= 0.000.
Table 3-3. Repeatability measurements of bone mineral density for total body and muscle volume (MV) of knee extensor and ankle plantar flexor muscles on two separate occasions.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>V1</th>
<th>V2</th>
<th>ICC</th>
<th>95% CI</th>
<th>P-value</th>
<th>Typical error</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD (g/cm$^3$)</td>
<td>1.26±0.1</td>
<td>1.26±0.1</td>
<td>0.996</td>
<td>0.987/0.999</td>
<td>0.000</td>
<td>7.0x10^{-3}</td>
</tr>
<tr>
<td>MV-Soleus (cm$^3$)</td>
<td>424.9±110.4</td>
<td>427.1±116.1</td>
<td>0.996</td>
<td>0.986/0.999</td>
<td>0.000</td>
<td>10.6</td>
</tr>
<tr>
<td>MV-MG (cm$^3$)</td>
<td>141.2±38.3</td>
<td>138.8±34.9</td>
<td>0.991</td>
<td>0.969/0.997</td>
<td>0.000</td>
<td>4.8</td>
</tr>
<tr>
<td>MV-LG (cm$^3$)</td>
<td>72.2±27.9</td>
<td>73.7±29.0</td>
<td>0.993</td>
<td>0.976/0.998</td>
<td>0.000</td>
<td>3.3</td>
</tr>
<tr>
<td>MV-DF (cm$^3$)</td>
<td>244.6±64.6</td>
<td>241.1±68.7</td>
<td>0.994</td>
<td>0.981/0.998</td>
<td>0.000</td>
<td>6.9</td>
</tr>
<tr>
<td>MV-VM (cm$^3$)</td>
<td>452.9±151.3</td>
<td>455.0±162.5</td>
<td>0.998</td>
<td>0.993/0.999</td>
<td>0.000</td>
<td>10.3</td>
</tr>
<tr>
<td>MV-VI (cm$^3$)</td>
<td>405.5±129.1</td>
<td>412.1±139.8</td>
<td>0.991</td>
<td>0.969/0.997</td>
<td>0.000</td>
<td>18.3</td>
</tr>
<tr>
<td>MV-VL (cm$^3$)</td>
<td>409.2±115.1</td>
<td>409.4±110.0</td>
<td>0.998</td>
<td>0.993/0.999</td>
<td>0.000</td>
<td>7.4</td>
</tr>
<tr>
<td>MV-RF (cm$^3$)</td>
<td>110.1±40.5</td>
<td>109.8±37.0</td>
<td>0.990</td>
<td>0.966/0.997</td>
<td>0.000</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD, BMD= Bone mineral density, MV= Muscle volume (cm$^3$), MG= medial head of gastrocnemius, LG= lateral head of gastrocnemius, DF= dorsiflexor muscles, VM= vastus medialis, VI= vastus intermedius, VL= vastus lateralis, RF= rectus femoris. V1= visit 1, V2= visit 2. ICC= Intra-class Correlation Coefficient, 95% Confidence Interval with lower/upper bound.
Table 3-4. Intra-observer repeatability for measuring the CSA from the same MRI images of the same leg muscles on two separate days.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Image 1</th>
<th>Image 1:2</th>
<th>ICC</th>
<th>95%CI</th>
<th>P-value</th>
<th>Typical error</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSA-Soleus (cm²)</td>
<td>22.5±5.6</td>
<td>22.4±5.5</td>
<td>0.999</td>
<td>0.997/1.000</td>
<td>.000</td>
<td>0.2</td>
</tr>
<tr>
<td>CSA-MG (cm²)</td>
<td>10.5±3.5</td>
<td>10.5±3.6</td>
<td>0.995</td>
<td>0.983/0.999</td>
<td>.000</td>
<td>0.3</td>
</tr>
<tr>
<td>CSA-LG (cm²)</td>
<td>3.7±2.4</td>
<td>3.7±2.5</td>
<td>0.995</td>
<td>0.983/0.999</td>
<td>.000</td>
<td>0.2</td>
</tr>
<tr>
<td>CSA-DF (cm²)</td>
<td>10.9±2.4</td>
<td>10.8±2.4</td>
<td>0.995</td>
<td>0.982/0.998</td>
<td>.000</td>
<td>0.2</td>
</tr>
<tr>
<td>CSA-VM (cm²)</td>
<td>22.0±6.9</td>
<td>22.1±6.9</td>
<td>1.000</td>
<td>0.999/1.000</td>
<td>.000</td>
<td>0.2</td>
</tr>
<tr>
<td>CSA-VI (cm²)</td>
<td>15.4±5.6</td>
<td>15.3±5.6</td>
<td>0.999</td>
<td>0.996/1.000</td>
<td>.000</td>
<td>0.2</td>
</tr>
<tr>
<td>CSA-VL (cm²)</td>
<td>17.2±7.8</td>
<td>17.2±7.9</td>
<td>0.999</td>
<td>0.997/1.000</td>
<td>.000</td>
<td>0.2</td>
</tr>
<tr>
<td>CSA-RF (cm²)</td>
<td>1.7±1.4</td>
<td>1.7±1.4</td>
<td>0.992</td>
<td>0.973/0.998</td>
<td>.000</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard deviation. ICC= Intra-class Correlation Coefficient, 95% Confidence Interval with lower /upper bound. Typical error= S.D \_diff/√2. CSAs= Cross sectional areas (cm²), MG= medial head of gastrocnemius, LG= lateral head of gastrocnemius, DF= dorsiflexor muscles, VM= vastus medialis, VI= vastus intermedius, VL= vastus lateralis, RF= rectus femoris.
3.4.2.1 Peak values of CSAs of MRI images on two occasions

There was no significant difference for the maximal CSAs for the same participant on two separate sessions (Table 3-5).

Table 3-5. Maximal values of CSAs for each muscle on two occasions.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Peak of CSAs-v1</th>
<th>Peak of CSAs-v2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus (cm$^2$)</td>
<td>32.2</td>
<td>33.4</td>
</tr>
<tr>
<td>MG (cm$^2$)</td>
<td>21.1</td>
<td>21.4</td>
</tr>
<tr>
<td>LG (cm$^2$)</td>
<td>16.2</td>
<td>16.1</td>
</tr>
<tr>
<td>DF (cm$^2$)</td>
<td>14.9</td>
<td>15.0</td>
</tr>
<tr>
<td>VM (cm$^2$)</td>
<td>42.3</td>
<td>42.2</td>
</tr>
<tr>
<td>VI (cm$^2$)</td>
<td>35.4</td>
<td>35.4</td>
</tr>
<tr>
<td>VL (cm$^2$)</td>
<td>49.8</td>
<td>49.9</td>
</tr>
<tr>
<td>RF (cm$^2$)</td>
<td>17.6</td>
<td>16.3</td>
</tr>
</tbody>
</table>

CSAs= Cross sectional areas (cm$^2$), MG= medial head of gastrocnemius, LG= lateral head of gastrocnemius, DF= dorsiflexor muscles, VM= vastus medialis, VI= vastus intermedius, VL= vastus lateralis, RF= rectus femoris. V1= visit 1, V2= visit 2. As only one reading was chosen, we could not express the data as mean ± SD for the above values.
3.4.3 Musculoskeletal function and gender (table 3-6)

Age, body mass and BMI did not differ significantly between males and females (P= 0.9, 0.17 and 0.84 respectively) but there was a significant difference in the participant's height (P= 0.006). Maximal isometric values for knee extensor muscles in all measured angles were significantly lower in females compared to males (P= 0.033, 0.042 and 0.042). There were no significant differences between genders for the maximal isometric muscle strength of ankle plantar flexor muscles at all three angles, (P= 0.089, 0.148 and 0.062). Knee extensor and ankle plantar flexor muscle volumes were significantly lower in both distal and proximal muscles except for RF between women and men. BMD did not differ significantly between healthy men and women P= 0.089.
Table 3-6. Comparison of musculoskeletal measurements of males and females.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Male (n=8)</th>
<th>Female (n=4)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31.8±7.8</td>
<td>32.0±8.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.7±7.8</td>
<td>1.5±5.8</td>
<td>0.006</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>82.1±8.2</td>
<td>65.9±18.1</td>
<td>0.17</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.9±2.2</td>
<td>26.5±5.6</td>
<td>0.84</td>
</tr>
<tr>
<td>MVC_knee_55º (Nm)</td>
<td>241.3±67.9</td>
<td>128.0±29.4</td>
<td>0.033</td>
</tr>
<tr>
<td>MVC_knee_70º (Nm)</td>
<td>281.0±95.1</td>
<td>147.2±32.2</td>
<td>0.042</td>
</tr>
<tr>
<td>MVC_knee_85º (Nm)</td>
<td>275.0±102.3</td>
<td>142.5±33.6</td>
<td>0.042</td>
</tr>
<tr>
<td>MVC_ankle_0º (Nm)</td>
<td>113.3±32.5</td>
<td>81.7±26.9</td>
<td>0.089</td>
</tr>
<tr>
<td>MVC_ankle_-5º (Nm)</td>
<td>125.1±33.4</td>
<td>87.2±32.6</td>
<td>0.148</td>
</tr>
<tr>
<td>MVC_ankle_-10º (Nm)</td>
<td>138.1±35.2</td>
<td>93.5±29.0</td>
<td>0.062</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>1.3±0.1</td>
<td>1.1±0.07</td>
<td>0.089</td>
</tr>
<tr>
<td>MV_Soleus (cm³)</td>
<td>480.4±69.2</td>
<td>304.1±74.1</td>
<td>0.011</td>
</tr>
<tr>
<td>MV_MG (cm³)</td>
<td>162.3±34.2</td>
<td>107.9±21.9</td>
<td>0.017</td>
</tr>
<tr>
<td>MV_LG (cm³)</td>
<td>87.5±23.0</td>
<td>45.8±18.1</td>
<td>0.011</td>
</tr>
<tr>
<td>MV_DF (cm³)</td>
<td>273.2±50.0</td>
<td>181.8±48.2</td>
<td>0.017</td>
</tr>
<tr>
<td>MV_VM (cm³)</td>
<td>536.2±115.0</td>
<td>286.8±48.3</td>
<td>0.007</td>
</tr>
<tr>
<td>MV_VI (cm³)</td>
<td>460.6±92.0</td>
<td>266.3±38.7</td>
<td>0.011</td>
</tr>
<tr>
<td>MV_VL (cm³)</td>
<td>449.5±101.5</td>
<td>304.6±58.6</td>
<td>0.017</td>
</tr>
<tr>
<td>MV_RF (cm³)</td>
<td>110.5±51.8</td>
<td>99.8±5.2</td>
<td>0.497</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. Descriptive statistics; Two independent sample test (Mann-Whitney U test) to assess differences between musculoskeletal measurements with gender (males & females); BMI= Body mass index (kg/m²); MVC= Maximal voluntary contraction (Nm); MV= Muscle volume (cm³); BMD= Bone mineral density (g/cm²).
3.5 Discussion

Reliable measurement tools are essential to measure muscle strength, size and BMD if they are to be used to identify abnormalities in populations with disease. The main findings of this study were that repeatability measurements for isometric knee extensor and ankle plantar flexor muscle strength and muscle volume and total body BMD were highly reliable. There were also significant differences in lower limb muscle strength and volume but not BMD between males and females.

ICC is the most common method for evaluating the agreement between repeated measurements (8). Agreement was very high (ICC-0.95 –0.98) for isometric maximal muscle contraction of the knee extensors and ankle plantar flexors which was in agreement with a previous study (8). Impellizzeri, Bizzini et al. (2008) found that the Cybex NORM dynamometer was highly reliable for isokinetic muscle strength, with higher agreement for knee extensors than knee flexors, and the ICC values were very high, between 0.95 and 0.98, particularly with slow compared to high speeds during quadriceps contractions (7).

Typical errors sensitive enough to detect changes within individuals and groups were too small for measuring maximal voluntary contraction measurements for both knee extensors and ankle plantar flexors. This was consistent with another study (8).

The quadriceps muscle is an essential muscle for everyday use, particularly for walking and for the performance of other physical activities, and its function has
been widely studied (25). There was an increase in torque production, with increasing knee flexion angles due to the fact that different knee angles result in changes to muscle fibre length and pennation angles, and therefore force production (25). There is a strong relationship between muscle fibre length, pennation angle and the exerted force (26, 27), and it has been reported that ankle plantar flexor strength increases when the ankle joint angle changes from full plantar flexion to 15° degree in dorsiflexion (28). Furthermore, when the person's muscle is stretched the excitability of motoneurones is increased and exerted torque is also increased (28). This is in agreement with the findings of the present study which show that the highest ankle plantar flexion isometric contraction is associated with the highest angles.

Recently, MRI has been deployed to measure total muscle and intramuscular tissue volumes (29). It is a highly reproducible, non-radiographic, non-invasive method for visualizing and measuring muscle CSAs and volumes (30). The intraobserver repeatability for measuring the CSA in a randomly chosen slice of the lower limb muscles (thigh and calf) from the same MRI images of the same leg on two separate days showed excellent reliability with an ICC of 0.99. The test-retest reliability for muscle volume assessment of the knee extensors and ankle plantar flexors and dorsiflexors was also excellent. This was in line with another recent study (30). The muscle volume of each separate muscle depends on the CSA measurement of each. There were large and significant differences in the muscle volume of the plantar flexor muscle group in comparison with others. However, there were no significant differences in the muscle volume between the
Kullberg, Brandberg et al. (2009) concluded that there was a strong relationship between DEXA, MRI and CT in whole body composition analysis (22). Indeed, in the present study we have found DEXA to be a quick, safe and highly reliable method to assess musculoskeletal mass (31).

In the present study, women had significantly lower muscle strength than men, which may be attributed to a difference in the intrinsic contractile properties of muscle, the stiffness of the muscle tendon unit and motor-neural muscular activation as well as a larger proportion of type II muscle fibres compared to females (32). The quadriceps muscle had a greater muscle size in males and can produce stronger muscle contractions than females (32). This study showed that there was no significant difference in RF volume, which is in contrast to another study showing that CSA of the RF differed significantly between males and females in both the relaxed and contracted positions (33). This variation further depends on physiological factors, such as the proportion of fat tissue around the muscle which is inversely associated with muscle activation and force production (33).

### 3.6 Conclusion

This study has shown that isokinetic dynamometer (Cybex), MRI and DEXA are highly reliable methods for analysing musculoskeletal structure and function.
3.7 References


CHAPTER IV. REDUCED LOWER LIMB MUSCLE STRENGTH AND VOLUME IN PATIENTS WITH TYPE 2 DIABETES IN RELATION TO NEUROPATHY, INTRAMUSCULAR FAT AND VITAMIN D LEVELS

Monirah Almurdhi, Neil Reeves, Frank Bowling, Andrew Boulton, Maria Jezierska and Rayaz A. Malik
Reduced Lower-Limb Muscle Strength and Volume in Patients With Type 2 Diabetes in Relation to Neuropathy, Intramuscular Fat, and Vitamin D Levels

OBJECTIVE
Muscle weakness and atrophy of the lower limbs may develop in patients with diabetes, increasing their risk of falls. The underlying basis of these abnormalities has not been fully explained. The aim of this study was to objectively quantify muscle strength and size in patients with type 2 diabetes mellitus (T2DM) in relation to the severity of neuropathy, intramuscular noncontractile tissue (IMNCT), and vitamin D deficiency.

RESEARCH DESIGN AND METHODS
Twenty patients with T2DM and 20 healthy control subjects were matched by age, sex, and BMI. Strength and size of knee extensor, flexor, and ankle plantar and dorsiflexor muscles were assessed in relation to the severity of diabetic sensorimotor polyneuropathy (DSPN), amount of IMNCT, and serum 25-hydroxyvitamin D (25(OH)D) levels.

RESULTS
Compared with control subjects, patients with T2DM had significantly reduced knee extensor strength (P = 0.003) and reduced muscle volume of both knee extensors (P = 0.045) and flexors (P = 0.019). Ankle plantar flexor strength was also significantly reduced (P = 0.001) but without a reduction in ankle plantar flexor (P = 0.23) and dorsiflexor strength (P = 0.45) muscle volumes. IMNCT was significantly increased in the ankle plantar (P = 0.006) and dorsiflexor (P = 0.005). Patients with DSPN had significantly less knee extensor strength than those without (P = 0.02) but showed no difference in knee extensor volume (P = 0.38) and ankle plantar flexor strength (P = 0.21) or volume (P = 0.96). In patients with <25 nmol/L versus ≥25 nmol/L 25(OH)D, no significant differences were found for knee extensor strength and volume (P = 0.32 vs. 0.18) and ankle plantar flexors (P = 0.58 vs. 0.12).

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 is related to the severity of peripheral neuropathy but not IMNCT or 25(OH)D level.
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, and limitations of joint flexibility and range of motion, ultimately affecting gait and whole-body movements [4–6]. Although weakness and atrophy of the distal muscles and decreased ankle mobility and strength have been documented in several studies and related to the severity of neuropathy [7–9], underlying mechanisms have not been explored. Previous studies did not perform a comprehensive assessment of muscle strength in relation to morphology and internal composition. Patients with diabetes and obesity have an increased amount of intramuscular noncontractile tissue (IMNCT), which is highly correlated with 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 alterations in strength, and because many patients with diabetes are obese, they may have larger muscle size but greater muscle atrophy due to diabetic neuropathy [7]. Previous studies have shown atrophy of the ankle plantar and dorsiflexor muscles and knee extensors in patients with diabetic neuropathy compared with patients without neuropathy and control subjects [2,4,6,8]. However, the effect on more-proximal leg muscles (knee extensors and flexors), which confer a major effect on postural stability and gait performance, has not been established. Indeed, maximal isometric muscle strength has been related directly to muscle cross-sectional area (CSA) [11–13].

A decline in 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 nonspecific musculoskeletal pain, which may be attributed to vitamin D deficiency [17]. The degree of vitamin D deficiency is currently categorized according to circulating levels of 25-hydroxyvitamin D (25OHD) such that an adequate level is defined as >75 nmol/L (>30 ng/mL), insufficient as 50–75 nmol/L (20–30 ng/mL), deficient as 25–50 nmol/L (10–20 ng/mL), and severely deficient as <25 nmol/L (<10 ng/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 in severe vitamin D deficiency [17]. Vitamin D receptor levels decline in elderly subjects [17,19,20], and vitamin D deficiency is associated with atrophy of skeletal muscle fibers (type III) and a decline in muscle strength, leading to 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 have been inversely correlated with obesity, diabetes, and high triglyceride levels [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 the present study was to investigate muscle strength deficits in distal and proximal extensors and flexors in the lower limbs of patients with type 2 diabetes and to relate these to muscle size, severity of peripheral neuropathy, IMNCT, and vitamin D deficiency.

**RESEARCH DESIGN AND METHODS**

Twenty patients with type 2 diabetes and 20 control subjects without diabetes were assessed at the muscle function laboratory of Manchester Metropolitan University (Manchester, U.K.). Individuals with severe musculoskeletal problems; neurological, orthopedic, or surgical problems; severe foot deformities; foot ulcers; and amputations or who were pregnant were excluded. The study was approved by the U.K. National Health Service ethics committee and local research ethics committees at the University of Manchester and the Manchester Metropolitan University, and written informed consent was obtained from all subjects before participation. This research adhered to the tenets of the Declaration of Helsinki.

**Assessment of Neuropathy**

All patients with diabetes underwent assessment of BMI, blood pressure, hBA1c, lipid profile (total cholesterol, LDL, HDL, triglycerides), albumin creatinine excretion ratio, estimated glomerular filtration rate, and 25OBD. Symptoms of diabetic polyneuropathy were assessed with the Neuropathy Symptom Profile. Neurological deficits were evaluated with the Simplified Neuropathy Disability Score, which comprises vibration perception, pin prick and temperature sensations, and presence or absence of ankle reflexes. Vibration perception threshold was tested with a Horwell Neurothesiometer (Scientific Laboratory Supplies, Wiford, Nottingham, U.K.). Cold and warm thresholds and cold- and warm-induced pain were established on the dorsolateral aspect of the foot by using a TSA-II Neurosensory Analyzer (Medoc Ltd., Ramat-Yishai, Israel). Electrodiagnostic studies were performed with a Dantec Keypoint system (Dan Tec Dynamics Ltd., Bristol, U.K.) equipped with a Defense Information Systems Agency temperature regulator to keep a constant limb temperature of 32–35°C. Sural sensory nerve amplitude, sural sensory nerve conduction velocity, and peroneal motor nerve conduction velocity and amplitude were assessed by a consultant neurophysiologist. Diabetic sensorimotor polyneuropathy (DSPN) was defined according to the Toronto criteria [24]. Control subjects were assessed only for vibration perception threshold and Neuropathy Disability Score.

All subjects were scanned with laser in vivo corneal confocal microscopy (Heidelberg Retina Tomograph III; Stock Cornea Module; Heidelberg Engineering GmbH, Heidelberg, Germany); all images were captured through the section mode of Heidelberg Eye Explorer software, and approximately six high-clarity images per subject were analyzed from the central sub-basal nerve plexus. Four parameters were established to assess corneal nerve fiber damage: corneal nerve fiber density (the total number of nerve fibers per square millimeter), corneal nerve branch density (the total number of nerve branches per square millimeter), and corneal nerve fiber length (the total length [mm] of all nerve fibers per square millimeter) within the area of the cornea and corneal nerve fiber density (the degree of nonlinearity of the nerve fibers). These parameters were quantified with a semiautomated, purpose-written, proprietary software (EC Metrics;
M. A. Dabbah, Imaging Science Biomedical Engineering, University of Manchester, Manchester, U.K.). Intraepidermal nerve fiber density was quantified in skin biopsy samples from the dorsum of the foot, using established techniques (25).

Isoinertial Dynamometer
Maximal isometric muscle strength for knee extensors and ankle plantar flexors was assessed with an isoinertial dynamometer (Cybex NORM; Cyber International, Ronkonkoma, NY). The dynamometer measures joint torque (in newton meters [Nm]) at the knee and ankle, which reflects the net forces acting around the respective joints and the internal tendon moment arm length. Because joint torque primarily reflects the force produced by the major muscle groups acting around the joints (knee and ankle extendors) and for the purpose of optimizing clinical understanding, we used the term muscle strength to refer to the measurement of joint torque.

Tests were performed at three different angles for both the knee and the ankle joints of the dominant leg. To test knee extensor joint torque, subjects were seated and secured on the chair of the dynamometer with their knees flexed at 90° (0° = full knee extension) and their hip angle at 85° (0° = supine position). Three maximal voluntary isometric contractions of the knee extensors were performed at three knee joint angles in random order: 85°, 70°, and 55° of knee flexion with a 2-min rest interval between contractions and the highest value recorded.

To test ankle plantar flexor joint torque, subjects were positioned prone on the dynamometer with the knee in full extension and the ankle secured to the footplate. Maximal voluntary isometric plantar flexor joint torque was assessed at three joint angles in random order: 0° (neutral position [i.e., right angle between footplate and tibia]), −5° dorsiflexion, and −10° dorsiflexion. Three maximal isometric contractions also were performed, and the highest value was recorded (Nm). Each maximum isometric contraction for both knee and ankle was held for ~3-4 s with a 60-s rest interval between contractions within each angle and a 2-min rest 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 subject, thereby taking into account slight variations in the muscle force-length relationship between groups.

Magnetic Resonance Imaging
A 0.25-T MRI peripheral scanner (G-scan; Esaote, Milan, Italy) was used to scan the upper and lower regions of the leg with a T1 gradient echo scanning sequence using the following parameters: field of view = 200 × 200 mm; matrix = 256 × 192 pixels; slice thickness = 10 mm; interslice 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 CSA of specific muscles were analyzed. Major exclusion criteria for MRI were 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, rectus femoris), knee flexors (semitendinosus, biceps femoris, semimembranosus), ankle plantar flexors (soleus, medial and lateral heads of gastrocnemius muscles), and ankle dorsiflexors (tibialis anterior, extensor digitorum longus) were analyzed by digitizing software (OsiRIS; 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 analyzed for each specific muscle to provide a representative and accurate muscle volume calculation, three subjects were randomly selected and all the available CSAs analyzed consecutively. The muscle volume calculated from all available slices was then compared against calculations from 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 the 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 ($\sum\text{CSAs cm}^2$) and multiplied by the distance between each muscle section d (m) to derive the muscle volume (cm$^3$) as shown in Eq. 1:

\[
\text{Muscle volume (cm}^3\text{)} = (\sum\text{CSAs cm}^2 \times d) \tag{Eq. 1}
\]

Intramuscular Noncontractile Tissue
The density of various tissues is reflected by a different MRI signal intensity. Connective tissue yields low signal intensity values; whereas 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 scan, it is possible to determine shifts in signal intensity, indicating changes in tissue composition. OsiRIS 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 muscle studied, and a frequency distribution of the signal intensity in that CSA was obtained. The signal intensity value with the highest frequency from the selected muscle CSA was recorded when this signal intensity value comprised >10% of the pixel number from the total number of pixels within that specific muscle CSA. Three different levels along the subject's leg were chosen (proximal, mid, distal) according to the 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 t test was used to test between-group differences in the measured variables. Pearson correlation coefficients were used to test the relationship between muscle strength and other parameters. Data are presented as mean ± SD unless otherwise stated.

With Eq. 2, we performed a power analysis before the study (a priori power calculation) by using the ankle joint strength (torque) results of previous studies (7,26):

\[
n = \frac{2 \times (\bar{E}_a + \bar{E}_p) \times \alpha}{\Delta^2} \tag{Eq. 2}
\]

The power analysis indicated that we needed 14 subjects in each group to detect a difference of 22 Nm between groups (~20% difference between groups), with an α-level of 0.05 and a β-level of 0.9 (i.e., power of 90%). To account for dropout and potential data
problems, we recruited 20 subjects into each group.

RESULTS
Subjects
Twenty healthy control subjects (13 male, 7 female) and 20 patients with type 2 diabetes (15 male, 5 female [8 with DSPN, 12 with no DSPN]) were assessed. Age, height, and BMI were matched in patients with diabetes compared with control subjects (Table 1). Patients had reasonable control of their glycemia and lipid levels and evidence of mild neuropathy based on neurological examination, quantitative sensory testing, neurophysiology, corneal confocal microscopy, and skin biopsy (Table 1).

Muscle Strength
Knee and ankle muscle strength (Nm/kg) was significantly lower in patients with type 2 diabetes compared with control subjects at three different angles. Knee strength at 55° (1.3 ± 0.4 vs. 2.1 ± 0.7; P = 0.002), 70° (1.3 ± 0.5 vs. 2.0 ± 0.8; P = 0.002), and 85° (1.3 ± 0.4 vs. 1.8 ± 0.6; P = 0.009) was significantly reduced in patients compared with control subjects. Ankle strength at 0° (0.6 ± 0.2 vs. 0.9 ± 0.3; P = 0.008), 15° (0.7 ± 0.2 vs. 1.04 ± 0.3; P = 0.003), and 30° (0.7 ± 0.5 vs. 1.1 ± 0.3; P = 0.001) was significantly reduced in patients compared with control subjects. Accordingly, the average knee extensor (1.3 ± 0.5 vs. 1.9 ± 0.7; P = 0.004) and ankle plantar flexor (0.6 ± 0.2 vs. 1.95 ± 0.3; P = 0.001) strength was significantly lower in patients compared with control subjects (Table 2).

Muscle Volume
Muscle volume for the knee extensors (P = 0.04) and flexors (P = 0.01) was significantly lower in patients with type 2 diabetes compared with control subjects (Table 2). No significant reduction was found in ankle plantar (P = 0.23) and dorsiflexor (P = 0.45) muscle volume between groups (Table 2).

Intramuscular Noncontractile Tissue
IMNCT was significantly increased in the soleus (P = 0.006), dorsiflexor (P = 0.005), and lateral gastrocnemius (P = 0.05) muscles in patients with type 2 diabetes compared with control subjects (Table 2 and Fig. 1). No significant differences were found in IMNCT in the knee extensors or knee flexors between groups (Table 2).

<table>
<thead>
<tr>
<th>Table 1—Subject clinical characteristics</th>
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<td>25OHD/BMI</td>
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<td>25OHD/BSA</td>
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<td>Cholesterol (mmol/L)</td>
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</tr>
<tr>
<td>CNFT (TC)</td>
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<td>EIMN (mm²/m²)</td>
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</table>

Data are mean ± SD unless otherwise indicated. BS, body surface area; CNB, coreal nerve branch density; CNF, coreal nerve fiber density; CNFL, coreal nerve fiber length; CNFT, coreal nerve fiber tortuosity; CT, cold threshold; IENFD, intraepidermal nerve fiber density; NA, not applicable; PMNAP, peroneal motor nerve amplitude; PMNAPV, peroneal motor nerve conduction velocity; SNAP, sural sensory nerve amplitude; SNVC, sural sensory nerve conduction velocity; TC, tortuosity coefficient; WT, warm threshold.

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

Low Versus Normal Vitamin D Levels
No significant difference was found in muscle strength (1.2 ± 0.1 Nm/kg vs. 1.3 ± 0.5 Nm/kg; P = 0.32) and volume (392.1 ± 427.3 cm³ vs. 1,122.7 ± 175.1 cm³; P = 0.18) of the knee extensors and ankle plantar flexor strength (0.6 ± 0.2 Nm/kg vs. 0.7 ± 0.2 Nm/kg; P = 0.58) and volume (582.6 ± 306.9 cm³ vs. 768.1 ± 160.5 cm³; P = 0.13) between patients with 25OHD levels <25 nmol/L and those with 25OHD levels >25 nmol/L.

Correlations
A significant correlation was found between knee extensor strength and knee extensor muscle volume (r = 0.57, P = 0.007) in patients with type 2 diabetes. No significant correlation was found
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 \( r \) ranged from \(-0.34\) to \(-0.02\) at the ankle and from \(-0.33\) to \(0.37\) at the knee, severity of DSPN \( r = -0.36 \) at ankle and \(-0.48\) at knee), or 25OHD level \( r = -0.12 \) at ankle and 0.13 at knee) among patients.

**CONCLUSIONS**

The findings show that patients with type 2 diabetes have reduced proximal and distal lower-limb muscle strength compared with age-matched control subjects, which agrees 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 the patients with diabetes. In contrast, although the plantar flexor muscle strength was reduced in the patients, no measurable muscle atrophy existed. This finding may be attributed to the increase in intramuscular fat, which may mask muscle atrophy. Essentially, because 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 a reduction in both muscle strength and muscle volume in the patients. The increase in intramuscular fat in the lower leg as opposed to the proximal knee extensors and flexors of these patients 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 type 2 diabetes 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 and resulting in insulin resistance of the skeletal muscle in patients with type 2 diabetes (28). Aging is also associated with increased IMNCT within muscles of the lower limb in patients with type 2 diabetes (29).

We 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; 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 gait impairment, increased incidence of falls, and severe injuries with hospitalization. Indeed, resistance training exercises can improve muscle strength and 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]. MRI allows for accurate quantification of muscle CSA and volume and allows one to differentiate muscle from fat, connective tissue, and bone [34]. In the current study, knee extensor and flexor muscle volume was significantly smaller, and there was a trend for a smaller distal plan- tar flexor muscle volume in the patients with type 2 diabetes. Muscle volume is of course associated with muscle strength and power production (14), as others have found [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 fascia, resulting in decreased flexibility of the ankle joint and limited dorsiflexion during walking [36]. We also found knee extensor muscle strength to be reduced significantly in patients with DSPN compared with 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 antiglutation muscules [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, and 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, the current study is the first to systematically examine differences in muscle function and structure in relation to vitamin D deficiency in patients with type 2 diabetes. Although we show that all patients had insufficient levels of 25(OH)D, a low level of 25(OH)D (<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 high considering the optimal recommendation is 0.8 (40). Some other variables fell 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 that no true differences existed between groups in these other variables and would not have been found in a much larger sample. In conclusion, this small but detailed study was adequately powered for the majority of variables examined. Potential confounders, such as differences in BMI and ethnicity between groups, may have had an impact on the findings. However, patients with type 2 diabetes showed 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 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.

Acknowledgments. The authors thank the staff at the musculoskeletal laboratory of Manchester Metropolitan University and the NIHR/Wellcome Trust Clinical Research Facility of Central Manchester University Hospitals NHS Foundation Trust for providing high-quality service and state-of-the-art facilities to carry out this research.

Funding. This study was funded by National Institutes of Health, National Institute of Neurological Disorders and Stroke grant 1R01NS042593-03NINDS and NPRP grant I-2000-385.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. M.M.A. contributed to the data analysis, statistical analysis, and writing of the manuscript. N.D.R., F.I.B., A.J.M.B., and M.L. contributed to the review and revision of the manuscript. 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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RESPONSE TO COMMENT ON ALMURDHI ET AL.

Reduced Lower-Limb Muscle Strength and Volume in Patients With Type 2 Diabetes in Relation to Neuropathy, Intramuscular Fat, and Vitamin D Levels. Diabetes Care 2016;39:441–447

Diabetes Care 2016;39:e184–e185 | DOI: 10.2337/dc16-0020

We thank Treiber et al. (1) for sharing their data in relation to a greater prevalence of painful neuropathy and slower walking speeds in their patients taking a statin. They suggest that some of the differences observed in our study (2) could be explained by treatment with statins. We have used our data to assess if statins may contribute to lower-limb weakness or muscle atrophy and neuropathy.

We have compared our study parameters between patients with type 2 diabetes mellitus (T2DM) on a statin (n = 16) to those who were not on a statin (n = 4). The interpretation of our data is limited and cautious because of the small number of patients not on a statin and also the lack of matching for age, weight, and BMI. Nevertheless, muscle strength of the lower limb in the knee extensors (P = 0.47) and ankle plantar flexors (P = 0.28) did not differ significantly. Muscle volumes for the knee extensors (P = 0.04) and flexors (P = 0.09) were lower, with no difference in the ankle plantar (P = 0.21) and dorsiflexor (P = 0.24) muscle volumes of T2DM patients on a statin compared with patients not on a statin.

Patients with diabetes on a statin were older, and it is well known that muscle mass declines as a result of aging due to a reduction in skeletal muscle fiber number, size, and length (3). Although it has been suggested that a reduction in muscle size can result in reduced motor neuron unit activation and decreased muscle force and power generation (3), this was not observed in our study (2). Another possible explanation is that the older individuals on a statin have a reduction in physical activity, which particularly affects the antigravity muscles such as the knee extensors. Reduced muscle size, reduced muscle activation capacity, and aging are of course highly correlated with reduced muscle strength (4), but in our study (2) there was no difference between those patients taking a statin compared with those not taking a statin.

The loss of muscle mass is also associated with diabetic neuropathy, and in our study (2) vibration perception threshold (P = 0.0001) and Neuropathy Disability Score (P = 0.09) were significantly higher, indicative of neuropathy in patients with diabetes on a statin compared with patients not on a statin, similar to the findings of Treiber et al. (1). Of course, whether or not a T2DM patient is on a statin will always be confounded by age, cardiovascular risk, and the presence of other microvascular complications (5). Furthermore, contrary to the studies cited by Treiber et al. (1), a recent large study has shown that treatment with statins may prevent the development of diabetic neuropathy (6). And, of course, whether or not patients are taking a statin depends on whether they can tolerate it, particularly in relation to vitamin D deficiency (7). A large prospective study is required to establish the potential relationship between statin use, muscle volume and strength, and walking ability and falls in diabetes.

Acknowledgments. This study was funded by National Institutes of Health National Institute of Neurological Disorders and Stroke Grant 5RO1NS46259-03NINDS and JDRF Grant 5-2002-165.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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and proportion of different fiber types studied in whole vastus lateralis muscle from 15- to 83-year-old men. J Neurol Sci 1988;84:275–294
CHAPTER V. DISTAL LOWER LIMB STRENGTH IS REDUCED IN PARTICIPANTS WITH IMPAIRED GLUCOSE TOLERANCE AND IS RELATED TO INCREASED INTRAMUSCULAR FAT AND VITAMIN D DEFICIENCY
Research: Complications

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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Accepted 7 June 2016

Abstract

Aim To quantify muscle strength and size in subjects with impaired glucose tolerance (IGT) in relation to intramuscular non-contracile 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-contracile 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-contracile 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-contracile tissue, small fibre neuropathy and vitamin D deficiency.

Diabet. Med. 00, 000-000 (2016)

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-contracile 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].

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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.

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, HbA1c, 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, Boldon, 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, Brissol, UK), equipped with a DSHA 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 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^2]); corneal nerve branch density (total number of nerve branches [number/mm^2]); corneal nerve fibre length (total length of all nerve fibres [mm/mm^2] 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®; 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 30-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 Axiosmager M2 microscope at 400 x magnification. Intraperiodal nerve fibre density was quantified according to established criteria and expressed as number/mm² [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, Essex, Italy). A T1 gradient echo scanning sequence was used with the following parameters: field of view = 200 x 200 mm; matrix = 256 x 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 pregnant; subjects in whom ferromagnetic foreign bodies were detected; and those with cardiac pacemakers/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.

Intromuscular non-contractile 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 α level of 0.05 and a β 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 ± 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. HbA1c 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).
Table 1: Clinical and demographic characteristics of the participants

<table>
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<td>25(OH)D/Body surface area</td>
<td>1.9 ± 0.17</td>
<td>2.1 ± 0.23</td>
<td>0.002</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>3.8 ± 0.6</td>
<td>4.4 ± 0.8</td>
<td>0.007</td>
</tr>
<tr>
<td>Cholesterol, mmol/l</td>
<td>5.5 ± 0.8</td>
<td>4.7 ± 1.4</td>
<td>0.04</td>
</tr>
<tr>
<td>HDL, mmol/l</td>
<td>2.1 ± 0.3</td>
<td>1.2 ± 0.4</td>
<td>0.18</td>
</tr>
<tr>
<td>LDL, mmol/l</td>
<td>3.2 ± 0.7</td>
<td>2.1 ± 1.2</td>
<td>0.002</td>
</tr>
<tr>
<td>Triglycerides, mmol</td>
<td>1.7 ± 0.9</td>
<td>2.2 ± 1.3</td>
<td>0.22</td>
</tr>
<tr>
<td>NDS (0–10)</td>
<td>1.4 ± 1.2</td>
<td>3.3 ± 3.4</td>
<td>0.01</td>
</tr>
<tr>
<td>VPT, Hz</td>
<td>4.4 ± 3.1</td>
<td>16.9 ± 11.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Cold threshold, °C</td>
<td>27.5 ± 2.0</td>
<td>24.9 ± 5.8</td>
<td>0.06</td>
</tr>
<tr>
<td>Warm threshold, °C</td>
<td>38.6 ± 2.7</td>
<td>41.2 ± 3.7</td>
<td>0.03</td>
</tr>
<tr>
<td>Sensory nerve conduction velocity, m/s</td>
<td>48.6 ± 4.5</td>
<td>45.8 ± 13.8</td>
<td>0.38</td>
</tr>
<tr>
<td>Sensory nerve conduction velocity, m/s</td>
<td>13.7 ± 7.2</td>
<td>14.6 ± 14.4</td>
<td>0.82</td>
</tr>
<tr>
<td>Sensory nerve conduction velocity, m/s</td>
<td>46.6 ± 4.7</td>
<td>41.4 ± 10.7</td>
<td>0.05</td>
</tr>
<tr>
<td>Sensory nerve conduction velocity, mV</td>
<td>3.3 ± 3.8</td>
<td>3.8 ± 1.8</td>
<td>0.04</td>
</tr>
<tr>
<td>Corneal nerve fibre density, number/mm²</td>
<td>1.7 ± 1.1</td>
<td>27.6 ± 8.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Corneal nerve fibre length, mm/mm²</td>
<td>94.9 ± 33.6</td>
<td>55.7 ± 35.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Corneal nerve fibre thickness, tortuosity coefficient</td>
<td>26.7 ± 3.7</td>
<td>21.9 ± 4.5</td>
<td>0.007</td>
</tr>
<tr>
<td>Corneal nerve fibre density, number/mm²</td>
<td>16.4 ± 2.7</td>
<td>18.6 ± 6.5</td>
<td>0.16</td>
</tr>
<tr>
<td>Intraperiadermal nerve fibre density, number/mm²</td>
<td>7.7 ± 2.0</td>
<td>6.7 ± 3.4</td>
<td>0.28</td>
</tr>
</tbody>
</table>

25(OH)D: 25-hydroxyvitamin D; ICT: impaired glucose tolerance; NDS, Neuropathy Disability Score; VPT, vibration perception threshold. Values are mean ± sd, unless otherwise indicated.

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 planatar 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 planatar 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 significance 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 planatar flexor muscle strength <2 vs ≥2 so from those in the control group, there were no significant differences in any measure of neuropathy. There was a significant correlation between ankle plantatar 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 plantatar 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 = 0.46; P = 0.04) and sural sensory nerve amplitude (r = -0.54; P = 0.01; r = -0.54; P = 0.01), respectively. There was a significant correlation between IMNCT in the soleus muscle and intraperiadermal 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 extensor with intraperiadermal 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 ± 0.4 Nm/kg vs 1.7 ± 0.5 Nm/kg; P = 0.80) or knee extensor (1153 ± 159 cm² vs 1167 ± 546 cm²;
Table 2 Muscle volume (cm$^3$) with percentage difference and statistical differences between subjects with impaired glucose tolerance and control subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>IGT</th>
<th>$P$</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vastus medialis</td>
<td>342 ± 99</td>
<td>407 ± 119</td>
<td>0.08</td>
<td>18</td>
</tr>
<tr>
<td>Vastus</td>
<td>342 ± 106</td>
<td>400 ± 95</td>
<td>0.60</td>
<td>5</td>
</tr>
<tr>
<td>Intermedius</td>
<td>369 ± 107</td>
<td>402 ± 97</td>
<td>0.31</td>
<td>9</td>
</tr>
<tr>
<td>Vastus lateralis</td>
<td>149 ± 48</td>
<td>122 ± 36</td>
<td>0.06</td>
<td>-17</td>
</tr>
<tr>
<td>Rectus femoris</td>
<td>1202 ± 323</td>
<td>1164 ± 492</td>
<td>0.77</td>
<td>-3</td>
</tr>
<tr>
<td>Semi-membranosus</td>
<td>228 ± 38</td>
<td>268 ± 88</td>
<td>0.11</td>
<td>17</td>
</tr>
<tr>
<td>Biceps femoris</td>
<td>288 ± 77</td>
<td>310 ± 84</td>
<td>0.41</td>
<td>7</td>
</tr>
<tr>
<td>Semitendinosus</td>
<td>152 ± 51</td>
<td>147 ± 60</td>
<td>0.43</td>
<td>9</td>
</tr>
<tr>
<td>Kne. flexors</td>
<td>669 ± 173</td>
<td>673 ± 291</td>
<td>0.97</td>
<td>0.3</td>
</tr>
<tr>
<td>Sartorius</td>
<td>419 ± 115</td>
<td>483 ± 316</td>
<td>0.08</td>
<td>15</td>
</tr>
<tr>
<td>Medial gastrocnemius</td>
<td>183 ± 53</td>
<td>198 ± 72</td>
<td>0.52</td>
<td>7</td>
</tr>
<tr>
<td>Lateral</td>
<td>106 ± 36</td>
<td>106 ± 46</td>
<td>0.98</td>
<td>0</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>710 ± 187</td>
<td>747 ± 273</td>
<td>0.62</td>
<td>5</td>
</tr>
<tr>
<td>Ankle plantar flexors</td>
<td>218 ± 50</td>
<td>255 ± 68</td>
<td>0.06</td>
<td>16</td>
</tr>
</tbody>
</table>

IGT, impaired glucose tolerance.

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

$P = 0.83$ and flexor (643 ± 161 cm$^2$ vs 678 ± 319 cm$^2$), $P = 0.76$ 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 (6.6 ± 0.1 Nm/kg vs. 9.0 ± 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 ± 226 cm$^2$ vs 717 ± 340 cm$^2$, $P = 0.81$) or dorsiflexor (226 ± 70 cm$^2$ vs 263 ± 68 cm$^2$, $P = 0.3$) 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 mose lower limb as a result of impaired 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.
**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-calf 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 participants with IGT. Note also thick subcutaneous fat layer in participants with IGT, especially in (b). VM, vastus medialis; VL, vastus intermedius; VL, vastus lateralis; RF, rectus femoris; BF, biceps femoris; ST, semitendinosus; SM, semimembranosus; DF, Dorsiflexors; SOL, soleus; LG, lateral gastrocnemius. Scale bar along the bottom or the left side of each image = 10 cm.

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].
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.

Funding sources
This study was funded by both a National Institute of Health Grant (R01 NS46259-03) NINDS and a Juvenile Diabetes Research Foundation Grant (5-2002-185).

Competing interests
None declared.

Acknowledgements
We thank the staff at the musculoskeletal laboratory at Manchester Metropolitan University and the NIHR/Wellcome Trust Clinical Research Facility in Central Manchester University Hospitals NHS Foundation Trust for providing a high-quality service and their state-of-the-art facilities to carry out the research. We gratefully acknowledge the following for undertaking neuropathy and conical confocal assessment: M. Ferdowsi, S. Azmi, J.N. Petropoulos, H. Fadavi, G. Pournaras, A. Marshall, M. Tavakoli, O. Ashgar and U. Alam (Centre for Endocrinology and Diabetes, Institute of Human Development, University of Manchester and the Manchester Royal Infirmary, Central Manchester Hospital Foundation Trust, Manchester, UK).

References


Supporting Information

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

Appendix S1. Supplementary methods.
Chapter VI. Altered Walking Strategy and Increased Unsteadiness in Subjects with Impaired Glucose Tolerance and Type 2 Diabetes Relates to Small Fibre Neuropathy but Not Vitamin D Deficiency

Author’s contribution: Monirah Almurdhi contributed in walking and balance assessments during gait analysis. She performed statistical analysis and wrote the manuscript, which constitutes the basis of this chapter.

Monirah Almurdhi, Steven Brown, Frank Bowling, Andrew Boulton, Maria Jeziorska, Rayaz A. Malik and Neil D. Reeves
6.1 Abstract

Objective: Alterations in walking strategy and increased fall risk are recognised in patients with T2DM but the underlying basis and natural history are not clear. This study investigated alterations in walking strategy and dynamic sway (unsteadiness) in subjects with IGT and T2DM in relation to the severity of the neuropathy and vitamin D levels.


Results: Ankle power during walking was lower in subjects with IGT (P= 0.02) and T2DM (P= 0.003) whilst ankle strength was lower only in T2DM (P= 0.01) compared to controls. Walking speed was preserved in subjects with IGT but was lower in patients with T2DM (P= 0.008). Step width (P= 0.005) and dynamic medio-lateral sway (P= 0.007) were significantly higher in subjects with IGT but was preserved in T2DM. Dynamic medio-lateral sway correlated with CNFL (P= 0.001) and CNBD (P= 0.001) but not VPT (P= 0.19). 25(OH)D was significantly reduced in subjects with IGT (P= 0.04) but did not correlate with any walking variables or measures of dynamic sway.

Conclusions: Early abnormalities in walking strategy and dynamic sway were evident in subjects with IGT whilst ankle strength, power and walking speed were more affected in those with T2DM. Unsteadiness correlated with small, not large,
fibre neuropathy, but there was no relationship between vitamin D deficiency and walking variables.

**Keywords:** Impaired glucose tolerance, diabetes, gait analysis, balance, neuropathy, vitamin D.
6.2 Introduction

Walking impairment is perceived to be a late complication of diabetes as it has been described primarily in patients with established neuropathy (1-5). However, a number of studies have shown walking impairment in diabetic patients before the onset of large fibre neuropathy (6-9). This suggests that either neuropathy may not be the only contributor to this abnormality or that small rather than large fibre neuropathy may be a contributing factor.

Peripheral neuropathy disturbs both sensory and motor nerve function of the lower extremities, resulting in biomechanical alterations in walking and balance, with an increased risk of falls and severe injuries (1, 5, 6, 10-13). Patients with diabetes have reduced ankle and knee joint strength and power during walking as a result of distal muscular weakness (3, 5). Therefore, muscle weakness and alterations to muscle structure may lead to altered walking ability even in patients without DN (6). Slower walking speed, shorter step length and wider step width have all been demonstrated in diabetic patients without neuropathy compared to controls (7-9). Indeed, we have recently shown reduced lower limb strength and altered structure in T2DM patients with minimal neuropathy (14).

Sensory ataxia due to large fibre neuropathy and loss of vibration sense and motor impairment with altered walking strategies (2, 7) are considered advanced manifestations of DN. Previously, increased dynamic medio-lateral sway leading to unsteadiness has been reported in patients with DN (15). Neuropathy can impair lower extremity function in patients with diabetes (11), and loss of vibration
perception correlates with impaired dynamic medio-lateral sway in patients with diabetes (15). Postural unsteadiness during quiet standing has also been associated with the severity of neuropathy in patients with diabetes (9, 11). Together, these abnormalities may predispose patients to an increased risk of falls and injury.

IGT is a state of minor perturbations in glucose which results in a small but not large fibre neuropathy (16). However, a study of 8 subjects with IGT showed impaired standing balance and trunk position sense (17). Furthermore, we have recently shown that distal lower limb muscle strength was reduced in subjects with IGT compared to controls. This could potentially impact upon gait and stability during normal walking (18).

To assess whether measures of early small fibre neuropathy contribute to impaired walking ability, we have quantified small fibre neuropathy using CCM and IENFD in subjects with IGT and T2DM with mild neuropathy.

Muscle weakness, impairment of balance and increased risk of falls have been associated with vitamin D deficiency during upright quiet standing and walking in healthy elderly subjects (19-21). Reduced serum levels of 25(OH)D can cause proximal muscle weakness, impaired dynamic sway during walking and hence an increased risk of falls (20-22). A vicious circle may ensue whereby reduced daily activity may lead to obesity and vitamin D deficiency (23, 24), and indeed, obesity is an important risk factor for the development of IGT.
In this study, we aimed to investigate the natural history of alterations in walking strategy and unsteadiness in subjects with IGT and T2DM in relation to small and large fibre neuropathy and vitamin D deficiency.

6.3 Methods

6.3.1 Participants

20 patients with T2DM (8 with mild peripheral neuropathy and 12 without neuropathy, based on Toronto criteria), 20 subjects with IGT (10 with and 10 without neuropathy) and 20 control subjects without diabetes and neuropathy were recruited and underwent assessment at the gait laboratory at Manchester Metropolitan University. The study was approved by the UK National Health Service (NHS) Ethics Committee and local research ethics committees at the University of Manchester and Manchester Metropolitan University. Written informed consent was obtained from all subjects prior to participation. Subjects with poor visual function, severe musculoskeletal problems, neurological, orthopaedic or surgical problems, severe foot deformities, foot ulcers, amputations, or those requiring an assistive device to walk were excluded from the study.
6.3.2 Clinical assessment

All patients with diabetes and subjects with IGT underwent assessment of BMI, blood pressure, HbA1c, lipid profile [total cholesterol, LDL, HDL and triglycerides, albumin creatinine excretion ratio, estimated glomerular filtration rate and 25(OH)D.

6.3.3 Assessment of neuropathy

Symptoms of DPN were assessed using the Neuropathy Symptom Profile. Neurological deficits were evaluated using the simplified NDS which comprises vibration perception, pin-prick, temperature sensation and presence or absence of ankle reflexes. VPT was tested using a Neurothesiometer (Horwell, Scientific Laboratory Supplies, Wilford, Nottingham, UK). CT and WT 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. SNAP, SNCV, PMNCV and PMNA were assessed by a consultant neurophysiologist. DSPN was defined according to the Toronto criteria (25). Control subjects underwent assessment of VPT and NDS to confirm the absence of neuropathy.
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/subjects were analysed from the central sub-basal nerve plexus. Four parameters were established to assess corneal nerve fibre damage: CNFD, defined as the total number of nerve fibres per mm$^2$ (no./mm$^2$); CNBD, the total number of nerve branches (no./mm$^2$); CNFL, the total length of all nerve fibres (mm/mm$^2$) within the area of the cornea; and CNFT, the degree of non-linearity of the nerve fibres. These parameters were quantified using semi-automated, purpose-written, proprietary software (CCMetrics®, M. A. Dabbah, Imaging Science Biomedical Engineering, University of Manchester, Manchester, UK). IENFD was quantified in skin biopsies from the dorsum of the foot using our established techniques as described previously (26).

6.3.4 Gait analysis

Participants were invited to the gait laboratory at Manchester Metropolitan University to assess their normal walking strategy. Participants walked at their self-selected speed over a 10m level ground walkway, stepping onto two of the three ground-embedded force platforms (Kistler, Winterthur, Switzerland) sampling at 1000Hz. Participant’s movement was assessed using a 10-camera motion-capture system (Vicon, Oxford, UK) sampling at 100Hz. Using a full-body
modified Helen-Hayes marker set, 52 reflective markers were firmly fixed onto specific anatomical landmarks of the participant’s body (upper and lower body segments). Participants walked in standardised shoes appropriate for patients with diabetes (MedSurg; Darco, Raisting, Germany) to control footwear conditions between participants and also to ensure that patients with diabetes walked with appropriate footwear. The mean data from four walking trials containing two force platform strikes with the left and two with the right foot were selected for further analysis.

6.3.4.1 Gait parameters

Ankle and knee joint strength and power (peak values) during walking and temporal-spatial parameters (walking speed, step width and step length) were quantified for each participant using Visual 3D software (C-motion Inc., MD, USA) by combining the force and motion data. The mean value for all parameters from four trials was calculated, taking into account data from both left and right legs, with the strength and power values normalised to participant’s body mass.

6.3.4.2 Dynamic sway during walking

To quantify balance impairments during walking, a variable we termed ‘dynamic sway’ was calculated. This parameter was applied previously in diabetic patients (15). Briefly, we describe here how it was calculated (Fig. 6-1), and in this section
we will define how this parameter was derived. The whole body CoM was calculated from the tracked marker data (motion analysis) using the kinematic model. Dempster’s segment parameter model (27) was used to calculate mass distribution for each body segment, allowing the overall CoM to be calculated. The CoP under the foot was calculated using the resultant ground reaction force measurements from the force platforms; it was calculated as a weighted average when the participant’s feet were in contact with two separate force platforms. Dynamic sway is a term which is used from this point on in the manuscript to define the separation distance between the CoM and CoP during walking in two planes: a frontal plane (medio-lateral dynamic sway) and a sagittal plane (anterior-posterior dynamic sway) (Fig. 6-1). The maximum range and mean dynamic sway (in medio-lateral and anterior-posterior planes) was measured during walking.
Figure 6-1. Calculation of dynamic sway. Dynamic sway (S), defined as the separation of the body centre-of-mass (grey circle) and the centre-of-pressure (grey triangle). Dynamic sway is shown in the sagittal (left image) and frontal (right image) planes, illustrating the separation in the anterior-posterior direction and medial-lateral directions, respectively. Also highlighted in the sagittal plane image is the resultant ground reaction force vector (grey arrow) and the ankle joint centre position (black cross): joint moments (strength) were calculated based on the relative position and magnitude of the ground reaction force from the joint centre, as well as taking into account the mass and acceleration of the relevant body segments.
6.3.5 Statistical analysis

A one-way ANOVA with post-hoc Bonferroni was used to test the differences between the three study groups (T2DM, IGT and control) for the measured variables. An independent samples Student’s $t$-test was used to assess differences between two groups for specific categories of variables: differences in walking and dynamic sway variables between patients with and without neuropathy and with lower and higher values of vitamin D ($25(\text{OH})\text{D} <25\text{nmol/L}$ vs $>25\text{nmol/L}$). Pearson’s Chi-square ($\chi^2$) test of independence and a Fisher’s Exact test were used, for example, to evaluate the association between the categorical variables (differences in gender and ethnicity between groups in Table 6-1). A Pearson’s correlation coefficient was used to assess the correlation between walking and dynamic sway variables. For all the comparisons, significance was accepted at $P<0.05$. All the data are expressed as mean ± SD unless otherwise stated. The statistical power for the majority of walking and dynamic sway variables (peak ankle plantar flexor and knee extensor strength and power, walking speed, stride and step length, step width, anterior-posterior dynamic sway and medio-lateral dynamic sway) in subjects with IGT and patients with T2DM was 0.80-1.00 (Table 6-3), which is very high considering an optimal recommendation is 0.8 (28). However, some variables did fall below this optimal threshold and the statistical power could have been limiting.
6.4 Results

6.4.1 Clinical assessment (table 6-1)

20 healthy control subjects (13 males and 7 females), 20 subjects with IGT (16 males and 4 females) and 20 patients with T2DM (15 males and 5 females) were assessed. The participants were well matched for age and height but body mass (P= 0.006) and BMI (P= 0.01) were significantly higher in subjects with IGT compared to controls. 25(OH)D and the 25(OH)D/BMI (P= 0.05) and 25(OH)D/BSA (P= 0.01) ratios were significantly lower in subjects with IGT compared to controls. HbA1c was significantly higher (P= 0.003) in patients with T2DM compared to control subjects. Total cholesterol and LDL cholesterol were significantly lower in subjects with IGT (P= 0.05, P= 0.001) and T2DM (P= 0.001, P= 0.001) compared to controls, respectively.
Table 6-1. Clinical, anthropometric and metabolic variables in control subjects, subjects with IGT and patients with T2DM.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>IGT</th>
<th>T2DM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No (male/female)</td>
<td>20(13/7)</td>
<td>20(16/4)</td>
<td>20(15/5)</td>
<td>0.56</td>
</tr>
<tr>
<td>DSPN (with/without)</td>
<td>NA</td>
<td>10/10</td>
<td>8/12</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>61.5±6.0</td>
<td>62.7±11.1</td>
<td>63.1±10.8</td>
<td>0.86</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.6±0.09</td>
<td>1.7±0.07</td>
<td>1.6±0.08</td>
<td>0.10</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>78.1±11.5</td>
<td>94.9±18.7</td>
<td>82.6±18.2</td>
<td>0.006</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.2±3.8</td>
<td>31.5±5.5</td>
<td>29.4±4.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>(2/18)</td>
<td>(5/15)</td>
<td>(9/11)</td>
<td>0.02</td>
</tr>
<tr>
<td>Duration of DM (years)</td>
<td>NA</td>
<td>NA</td>
<td>14.9 ± 9.9</td>
<td></td>
</tr>
<tr>
<td>25(OH)D (nmol/L)</td>
<td>78.9±48.8</td>
<td>50.8±34.8</td>
<td>72.6±43.5</td>
<td>0.10</td>
</tr>
<tr>
<td>25(OH)D/BMI</td>
<td>2.9±1.9</td>
<td>1.6±1.1</td>
<td>2.5±1.6</td>
<td>0.04</td>
</tr>
<tr>
<td>25(OH)D/BSA</td>
<td>1.91±0.1</td>
<td>2.1±0.2</td>
<td>1.95±0.3</td>
<td>0.008</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.6±0.1</td>
<td>6.0±0.6</td>
<td>7.3±1.5</td>
<td>0.003</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.5±0.8</td>
<td>4.7±1.4</td>
<td>4.0±0.7</td>
<td>0.000</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>2.1±3.0</td>
<td>1.2±0.4</td>
<td>1.37±0.9</td>
<td>0.2</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>3.2±0.7</td>
<td>2.1±1.2</td>
<td>1.6±0.6</td>
<td>0.000</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.7±0.7</td>
<td>2.2±1.4</td>
<td>2.0±1.5</td>
<td>0.55</td>
</tr>
</tbody>
</table>

IGT: Impaired glucose tolerance; T2DM: Type 2 diabetes mellitus; DSPN: Diabetic sensori-motor polyneuropathy; BMI: Body mass index; BSA: Body surface area; 25(OH)D: 25-hydroxy-vitamin D; HbA1c: Glycated Haemoglobin; HDL: High density lipoprotein; LDL: Low density lipoprotein. Significant differences between groups as a result of ANOVA (final column) and as a result of Bonferroni post-hoc (p value in superscript) compared to controls are indicated in bold.
6.4.2 Neuropathy assessments (table 6-2)

NDS and VPT were significantly higher in both IGT (P = 0.02, P = 0.001) and T2DM (P = 0.04, P = 0.02) compared to controls respectively. Cold sensory thresholds and peroneal and sural nerve conduction velocity and amplitudes did not differ between groups. Warm sensory thresholds were higher in T2DM compared to controls (P = 0.03), and CNFD was significantly lower in subjects with IGT (P = 0.004) and patients with T2DM (P = 0.004) compared to controls. CNBD (P = 0.002) was significantly lower only in subjects with IGT (P = 0.002) whereas CNFT was significantly higher in patients with T2DM (P = 0.047) compared to controls. IENFD did not differ between groups.
Table 6-2. Neuropathy measures in control subjects, subjects with IGT and patients with T2DM.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>IGT</th>
<th>T2DM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDS (0-10)</td>
<td>1.1±1.2</td>
<td>3.3±3.4(^{(0.02)})</td>
<td>3.1±2.5(^{(0.04)})</td>
<td>0.014</td>
</tr>
<tr>
<td>VPT (Hz)</td>
<td>6.4±3.0</td>
<td>16.5±11.6(^{(0.001)})</td>
<td>13.7±8.6(^{(0.02)})</td>
<td>0.001</td>
</tr>
<tr>
<td>CT (°C)</td>
<td>27.5±2.1</td>
<td>24.9±5.7</td>
<td>26.1±3.2</td>
<td>0.12</td>
</tr>
<tr>
<td>WT (°C)</td>
<td>38.6±2.7</td>
<td>41.2±3.7</td>
<td>41.6±4.8(^{(0.05)})</td>
<td>0.03</td>
</tr>
<tr>
<td>SNCV (m/s)</td>
<td>48.6±4.5</td>
<td>45.8±13.6</td>
<td>47.4±4.4</td>
<td>0.58</td>
</tr>
<tr>
<td>SNAP (μV)</td>
<td>13.7±7.2</td>
<td>14.5±14.8</td>
<td>11.6±5.6</td>
<td>0.62</td>
</tr>
<tr>
<td>PMNCV (m/s)</td>
<td>46.6±4.7</td>
<td>41.4±10.7</td>
<td>42.2±7.05</td>
<td>0.08</td>
</tr>
<tr>
<td>PMNA (mV)</td>
<td>5.3±1.8</td>
<td>3.8±1.8</td>
<td>4.15±2.5</td>
<td>0.07</td>
</tr>
<tr>
<td>CNFD (no/mm(^2))</td>
<td>35.9±5.1</td>
<td>27.6±8.2(^{(0.004)})</td>
<td>27.7±9.1(^{(0.004)})</td>
<td>0.001</td>
</tr>
<tr>
<td>CNBD (no/mm(^2))</td>
<td>94.9±33.6</td>
<td>55.7±35.8(^{(0.005)})</td>
<td>93.9±41.5</td>
<td>0.002</td>
</tr>
<tr>
<td>CNFL (mm/mm(^2))</td>
<td>26.7±3.8</td>
<td>21.8±6.5</td>
<td>23.1±8.6</td>
<td>0.06</td>
</tr>
<tr>
<td>CNFT (TC)</td>
<td>16.4±2.7</td>
<td>18.6±6.5</td>
<td>20.2±4.3(^{(0.04)})</td>
<td>0.05</td>
</tr>
<tr>
<td>IENFD (no/mm)</td>
<td>7.7±2.0</td>
<td>6.7±3.4</td>
<td>7.6±5.4</td>
<td>0.70</td>
</tr>
</tbody>
</table>

IGT: Impaired glucose tolerance; T2DM: Type 2 diabetes mellitus; 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; PMNA: Peroneal motor nerve amplitude; CNFD: Corneal nerve fibre density; CNBD: Corneal nerve branch density; CNFL: Corneal nerve fibre length; CNFT: Corneal nerve fibre tortuosity; IENFD: Intraepidermal nerve fibre density. Significant differences between groups as a result of ANOVA (final column) and as a result of Bonferroni post-hoc (p value in superscript) compared to controls are indicated in bold.
6.4.3 Peak ankle and knee joint strength and power during walking (table 6-3)

During walking, peak ankle plantar flexion strength was significantly lower (P=0.01) in patients with T2DM whereas ankle plantar flexion power was significantly lower in subjects with IGT (P=0.02) and patients with T2DM (P=0.003) compared to controls. There were no significant differences in the peak knee extension strength and power between groups.

6.4.4 Temporal-spatial gait parameters (table 6-3)

Patients with T2DM displayed a significantly slower walking speed (P=0.008) compared to control subjects. There was no difference in stride or step length between either group except for a significantly greater step width (P=0.005) in subjects with IGT compared to controls.

6.4.5 Dynamic sway (table 6-3)

During walking, dynamic medio-lateral sway (P=0.007) was higher and posterior maximal movement (P=0.000) was lower in subjects with IGT compared to controls. Only dynamic anterior-posterior sway (P=0.02) was significantly lower in patients with T2DM compared to controls.
Table 6-3. Walking parameters in control subjects, subjects with IGT and patients with T2DM.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls</th>
<th>IGT</th>
<th>T2DM</th>
<th>P-value</th>
<th>Statistical power (IGT)</th>
<th>Statistical power (T2DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak ankle strength</td>
<td>1.2±0.2</td>
<td>1.1±0.2</td>
<td>1.1±0.2&lt;sup&gt;(0.01)&lt;/sup&gt;</td>
<td>0.01</td>
<td>0.82</td>
<td>0.89</td>
</tr>
<tr>
<td>Peak ankle power (W/kg)</td>
<td>3.0±0.8</td>
<td>2.4±0.6&lt;sup&gt;(0.03)&lt;/sup&gt;</td>
<td>2.2±0.6&lt;sup&gt;(0.003)&lt;/sup&gt;</td>
<td>0.002</td>
<td>0.99</td>
<td>1.00</td>
</tr>
<tr>
<td>Peak Knee strength</td>
<td>0.7±0.2</td>
<td>0.7±0.2</td>
<td>0.6±0.3</td>
<td>0.80</td>
<td>0.80</td>
<td>0.26</td>
</tr>
<tr>
<td>Peak Knee power (W/kg)</td>
<td>1.0±0.5</td>
<td>0.8±0.4</td>
<td>0.8±0.6</td>
<td>0.16</td>
<td>0.88</td>
<td>0.66</td>
</tr>
<tr>
<td>Temporal-spatial gait parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking speed (m/s)</td>
<td>1.4±0.2</td>
<td>1.3±0.3</td>
<td>1.2±0.2&lt;sup&gt;(0.008)&lt;/sup&gt;</td>
<td>0.008</td>
<td>0.65</td>
<td>1.00</td>
</tr>
<tr>
<td>Stride length (cm)</td>
<td>149.4±13.7</td>
<td>140.8±25.6</td>
<td>138.1±16.6</td>
<td>0.15</td>
<td>0.62</td>
<td>0.81</td>
</tr>
<tr>
<td>Step Length (cm)</td>
<td>74.7±6.8</td>
<td>70.4±12.8</td>
<td>69.0±8.2</td>
<td>0.15</td>
<td>0.62</td>
<td>0.96</td>
</tr>
<tr>
<td>Step width (cm)</td>
<td>10.9±2.5</td>
<td>13.5±2.4&lt;sup&gt;(0.00)&lt;/sup&gt;</td>
<td>12.0±2.6</td>
<td>0.006</td>
<td>1.00</td>
<td>0.61</td>
</tr>
<tr>
<td>Dynamic Sway (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ant. Max</td>
<td>27.3±6.7</td>
<td>26.2±7.2</td>
<td>23.5±5.7</td>
<td>0.17</td>
<td>0.17</td>
<td>0.86</td>
</tr>
<tr>
<td>Mean AP</td>
<td>14.4±3.2</td>
<td>13.6±3.4</td>
<td>11.9±1.8&lt;sup&gt;(0.03)&lt;/sup&gt;</td>
<td>0.02</td>
<td>0.29</td>
<td>1.00</td>
</tr>
<tr>
<td>AP Range</td>
<td>46.8±10.9</td>
<td>44.1±12.3</td>
<td>40.6±11.0</td>
<td>0.22</td>
<td>0.27</td>
<td>0.81</td>
</tr>
<tr>
<td>Post. Max</td>
<td>28.0±2.8</td>
<td>17.7±5.5&lt;sup&gt;(0.00)&lt;/sup&gt;</td>
<td>25.7±2.8</td>
<td>0.000</td>
<td>1.00</td>
<td>0.98</td>
</tr>
<tr>
<td>ML mean</td>
<td>5.0±2.5</td>
<td>8.1±3.7&lt;sup&gt;(0.007)&lt;/sup&gt;</td>
<td>6.4±2.3</td>
<td>0.009</td>
<td>1.00</td>
<td>0.78</td>
</tr>
<tr>
<td>ML Range</td>
<td>12.7±1.7</td>
<td>13.6±0.3</td>
<td>15.2±9.3</td>
<td>0.37</td>
<td>0.99</td>
<td>0.65</td>
</tr>
<tr>
<td>ML. Max</td>
<td>7.4±3.5</td>
<td>9.4±4.9</td>
<td>9.0±2.8</td>
<td>0.26</td>
<td>0.69</td>
<td>0.73</td>
</tr>
</tbody>
</table>

IGT: Impaired glucose tolerance; T2DM: Type 2 diabetes mellitus; W: Watt; Ant: Anterior; AP: Anterior posterior; Post: Posterior; ML: Medio-lateral; Max:
Maximum. Significant differences between groups as a result of ANOVA and as a result of Bonferroni post-hoc (p value in superscript) compared to controls are indicated in bold. Statistical power for each group is indicated.

6.4.6 Peripheral neuropathy vs no peripheral neuropathy

There were no significant differences in ankle plantar flexion strength (1.1±0.1 vs 1.2±0.1 Nm/kg; P= 0.87) and power (2.5±0.6 vs 2.6±0.7 W/kg; P= 0.54), knee extension strength (0.7±0.2 vs 0.6±0.1 Nm/kg; P= 0.29) and power (0.8±0.6 vs 0.9±0.2 W/kg 43; P= 0.55), walking speed (1.3±0.2 vs 1.3±0.2 m/s; P= 0.77), step width (13.0±2.8 vs 11.7±2.6 cm; P= 0.11), step length (148.4±17.9 vs 140.1±19.9 cm; P= 0.11) and dynamic medio-lateral sway (14.9±8.0 vs 13.4±4.3 cm; P= 0.46) in subjects with (n= 19) compared to subjects without (n= 41) peripheral neuropathy, respectively.

6.4.7 Low (<25nmol/L) vs normal vitamin D (>25nmol/L)

During walking, only step length (70.5±10.3 vs 75.1±5.9 cm; P= 0.05) was significantly lower in subjects with a 25(OH)D levels <25nmol/L compared to subjects with >25nmol/L. There was no significant difference in ankle plantar flexion strength (1.1±0.1 vs 1.2±0.1 Nm/kg; P= 0.22) and power (2.3±0.5 vs 2.6±0.74 W/kg; P= 0.12), knee extensor strength (0.5±0.2 vs 0.6±0.2 Nm/kg; P=
0.14) and power (0.6±0.4 vs 0.9±0.5 W/kg; P= 0.11), walking speed (1.4±0.2 vs 1.3±0.2 m/s; P= 0.13), step width (12.1±1.8 vs 12.1±2.8 cm; P= 0.92) and dynamic medio-lateral sway (9.7±3.9 vs 8.4±3.8 cm; P= 0.36) between subjects with low compared to normal 25(OH)D. However, dynamic medio-lateral sway was higher in patients with T2DM (12.5±1.9 vs 8.1±2.4 cm; P= 0.01) but not subjects with IGT (8.2±4.9 vs 9.7±5.0 cm; P= 0.62) comparing those with 25(OH)D levels <25nmol/L compared to >25nmol/L respectively.

### 6.4.8 Correlations

There were low-to-moderate correlations between walking speed with ankle power (r= 0.28; P= 0.03) and with ankle plantar flexor strength (r= 0.29; P= 0.02) and also between dynamic medio-lateral sway with CNFL (r= 0.406; P= 0.001) and CNBD (r= 0.436; P= 0.001). However, there was no significant correlation between medio-lateral sway and VPT (r= 0.17; P= 0.19).

### 6.5 Discussion

This is the first study to show an early alteration in the natural walking strategy and dynamic sway in subjects with IGT which is comparable to patients with T2DM. This challenges the belief that balance impairment during walking only occurs in patients with diabetes and advanced large fibre neuropathy (15). In the present study, we also show early small fibre deficits in subjects with IGT and patients with mild-to-moderate DN and a minimal abnormality in vibration
perception and electrophysiology. Furthermore, we show that small rather than large fibre abnormalities are more strongly associated with balance impairment.

Whilst we confirm previous findings of a reduction in ankle joint strength and power during walking in patients with T2DM (2, 3, 29), we also show that subjects with IGT display a reduction in ankle joint power during walking. These findings are further supported by our recent data showing a significant weakness of the distal ankle plantar flexors in patients with T2DM (14) and subjects with IGT (18).

It has previously been shown that patients with DN have a slower self-selected walking speed, smaller step length and greater step width compared to controls (2, 8, 29). In the present study, whilst walking velocity, stride length and step length were preserved, the stride width was greater in subjects with IGT, suggesting that this may be an early defect. Previously, we have observed a greater step width in patients with severe DN compared to controls (15). This was not found in the diabetic patients in the present study as they had evidence of very mild neuropathy. However, the IGT group with an equivalent severity of neuropathy had adopted an increased step width. This may be attributed to the greater BMI in this group. Indeed, whilst a greater step width has been traditionally regarded as a way for diabetic patients to increase their base of support (30), we recently reported a positive correlation between step width and medio-lateral dynamic sway (15). In other words, because walking requires the transfer of body weight from one leg to another, a wider stance may actually impair balance during walking because the body’s CoM needs to be moved
further from side-to-side and hence this may be an early sign of walking impairment in subjects with IGT. In patients with T2DM, however, only walking speed was altered. This highlights that alterations to gait strategy begin early, even in subjects with IGT, representing a more “cautious” walking strategy commonly observed in diabetic patients with neuropathy (8, 29).

The lower ankle strength and power observed during walking in subjects with IGT and diabetes may be one of the main underlying factors explaining the slower walking speed (3), particularly ankle power which reflects aspects of both force and speed (1, 4, 12, 15, 29, 31). Indeed, we observed a positive correlation between walking speed and ankle power, and also between walking speed and ankle plantar flexor strength during walking. The slower walking speed observed in patients with IGT and T2DM may well be a compensatory strategy to lower the strength demands of walking as reported in previous studies in patients with diabetes (19). Furthermore, the reduced step length observed in subjects with IGT and T2DM in the present study constitutes a “biomechanical strategy” that helps to reduce the strength required from knee and ankle muscles by reducing the flexion (and therefore the required torque) of these joints. Diminished cognitive function may be another possible reason for a reduced walking speed (1, 6, 23), which has been related to an increased risk of unsteadiness during walking (6).

Reduced ankle strength and power during walking may play a role in unsteadiness, which can be quantified by measuring dynamic medio-lateral sway. Indeed, we have found that dynamic medio-lateral sway was already
higher in subjects with IGT during walking indicating greater unsteadiness, in agreement with our recent findings in patients with diabetic peripheral neuropathy (15). This is in line with previous findings of impaired postural sway during upright standing balance tests in subjects with IGT (17). In the IGT group of the present study, we found a higher mean dynamic medio-lateral sway, which means on average they demonstrated more side-to-side sway, whereas the T2DM group displayed a greater range of dynamic medio-lateral sway, which means they periodically allowed greater side-to-side sway, indicating more variability and a potentially more “dangerous” situation in terms of the risk of falling for the T2DM group. DPN may be an important factor which negatively impacts on balance during walking (4, 15, 32). Indeed, a recent study has shown that patients with diabetic neuropathy have a disturbance in postural sway during upright standing (33). The presence of peripheral neuropathy may be a potential explanation for the increased medio-lateral sway observed in subjects with IGT in the present study. However, our patients displayed evidence of minimal large fibre neuropathy. Traditionally, it has been suggested that quite marked impairment in sensation and proprioception is required to cause walking alterations (1, 3, 4, 12, 29). However, we now show that there are significant alterations with minimal evidence of large fibre neuropathy.

We have previously shown an association between loss of vibration perception and dynamic medio-lateral sway during walking in patients with diabetes (15). However, in the present study we have found no correlation between VPT and medio-lateral sway. A possible reason for this is that the majority of patients had
very minimal large fibre neuropathy. However, a novel finding in the present study is the much stronger association between measures of small fibre neuropathy determined using CCM with dynamic sway compared to large fibre neuropathy assessed using VPT and electrophysiology. The mechanistic basis for this association between dynamic sway and small fibre pathology requires further study, especially as there was no association with IENFD in the foot. We have also shown that subjects with IGT have smaller dynamic anterior-posterior sway, which reflects the shorter step length taken by subjects with IGT, comparable to previous studies in patients with diabetes (8, 15, 29). The smaller posterior maximum anterior-posterior sway is likely explained by subjects with IGT not allowing their body CoM to remain as far back compared to the controls and T2DM groups when transferring over to the leading leg during walking. This is likely because the joint moments (strength) associated with keeping the CoM any further back would be very high due to the flexed joints and high body mass of the IGT group.

Previous studies have reported that vitamin D deficiency is related to postural instability during quiet standing and an increased risk of falling in elderly subjects (34). However, in the present study we found no relationship between vitamin D deficiency and walking strategy or unsteadiness. Although the vitamin D levels were not markedly reduced, another possible reason is that vitamin D deficiency affects proximal pelvis and hip muscles more than distal muscles of the lower limb (21, 34, 35). The present study found that patients with T2DM and low vitamin D had significantly higher dynamic medio-lateral sway compared to
patients with adequate levels of vitamin D, suggesting that vitamin D deficiency may have a role in balance impairment in T2DM. Previously, healthy subjects with inadequate vitamin D (<50nmol/L) have been shown to have a higher postural sway during upright quiet standing compared to those with an adequate level of vitamin D. Also, subjects with severe vitamin D deficiency (<25nmol/L) suffer from decreased muscle strength particularly in proximal muscles, increased postural sway during quiet standing and an increased risk of falls (36).

6.6 Conclusion

We have shown that alterations to walking strategy and increased dynamic sway occur in subjects with IGT and T2DM and are related to small rather than large fibre neuropathy but not vitamin D deficiency. Longitudinal and mechanistic studies are required to better understand the evolution and consequences of these abnormalities.
6.7 References


CHAPTER VII. DISCUSSION
7.1 Introduction

The International Diabetes Federation (IDF) estimates that diabetes will affect 552 million people globally by 2030 (1). DPN is a major long-term complication that is associated with increased morbidity and mortality and affects ~50% of all diabetic patients (2-8). IGT is an intermediate metabolic disorder that will lead to diabetes in at least 1/3 of subjects unless a change in diet or lifestyle is implemented (9). Most previous studies assessing motor complications in diabetes have studied patients with advanced neuropathy and have not attempted to relate alterations in muscle function to motor structure and abnormalities in gait. Few studies have investigated motor impairment in subjects with IGT and patients with T2DM, particularly in relation to detailed measures of peripheral neuropathy and vitamin D deficiency.

Vitamin D deficiency is common in patients with diabetes compared to healthy subjects (10). Previous studies have shown lower levels of vitamin D in patients with obesity (11, 12), T2DM and subjects with IGT (13, 14). The exact mechanism(s) linking vitamin D deficiency and T2DM are unclear (11). Subjects with vitamin D deficiency suffer from musculoskeletal pain and myopathy, particularly in the proximal muscles of the lower extremities (15-18). This musculoskeletal pain reduces physical activity (19-22) with a reduction in skeletal muscle strength at the hip flexors, knee extensors and flexors resulting in abnormalities in walking pattern and dynamic medio-lateral sway, and an increased risk of falls and hip fracture in otherwise healthy elderly subjects (19-
25). The muscle weakness and atrophy of the lower extremities has been related to the severity of vitamin D deficiency (21, 22, 25). Thus, both vitamin D deficiency and DN contribute to reduced muscle strength and size, disturbance of balance during walking, impaired normal walking strategy and an increased risk of falls. There are no studies that have investigate the relationship between vitamin D deficiency and motor dysfunction of the lower limbs in patients with T2DM and mild neuropathy and subjects with IGT. This thesis investigates the hypothesis that patients with mild DN but with inadequate levels of vitamin D will have a significant reduction in muscle strength and size of the lower limbs, altered biomechanical walking strategy and increased dynamic medio-lateral sway during walking than diabetic patients without neuropathy and adequate levels of vitamin D.

7.2 Repeatability of maximal isometric muscle strength, lower limb muscle volume and bone mineral density (chapter 3)

Minimal to marked muscle weakness can be assessed using the isokinetic dynamometer to identify reduced distal muscle strength particularly at the knee and ankle joints (26). Muscle volume of the lower extremities can be measured using peripheral MRI in healthy subjects and patients with motor problems. It also provides high quality resolution of the architecture of the skeletal muscle which allows quantification of the CSA of each single muscle (27, 28). Low levels of 25(OH)D concentrations can cause musculoskeletal dysfunction in healthy
subjects with diabetes mellitus (29). BMD can easily be evaluated using DEXA (30). To ensure that these instruments are reliable in quantifying these different parameters, a pilot study was undertaken in healthy control subjects to establish intra-observer repeatability, agreement and symmetry of isometric muscle contraction of knee extensors and ankle plantar flexor muscles using an isokinetic dynamometer (Cybex); muscle volume of the lower extremity muscles using MRI; and BMD measurement using DEXA. This study showed that the isokinetic dynamometer, MRI and DEXA scanners had small values of typical errors and good reliability when evaluated using the ICC coefficient (31, 32). This study also showed that increased elongation of skeletal muscle leads to increased stimulation of motoneurones and consequently increased force production (33-35).

MRI can reliably quantify muscle area and detect pathological changes in the musculoskeletal system affecting the muscles, connective tissue, fat and bone (28, 36). This study also found excellent reliability with an ICC 0.99 for intra-observer repeatability of CSA assessment of each single muscle of the lower limb in randomly selected MRI images taken on two separates days by the same examiner. DEXA is also a quick method to assess bone structure (30, 37). Previous studies have shown that muscle strength depends on age, height and body mass (38) and gender with a 5-fold difference between males and females (39). Thus, in the present study young males were stronger and had larger muscle bulk of the lower limb than females (39, 40), whilst increasing age reduces muscle strength and size in healthy subjects in both genders (37, 41,
This study has confirmed that isokinetic dynamometer, MRI and DEXA scanners are highly reliable, safe and sensitive tests to evaluate muscle strength, size and bone density in subjects with IGT and patients with T2DM with or without neuropathy.

7.3 Reduced lower limb muscle strength and volume in patients with type 2 diabetes in relation to neuropathy, intramuscular fat and vitamin D levels (chapter 4)

Patients with DN have marked muscle weakness and atrophy in the distal muscles of the lower limb that has been related to the severity of neuropathy (43-45). An increase in IMNCT is highly associated with insulin resistance and muscle weakness of the ankle and knee joints in patients with diabetes (46-48). Decreased muscle strength and size, with increased infiltration of intramuscular fat and reduced physical activity in healthy elderly subjects occurs in those with vitamin D deficiency (49, 50). Patients with diabetes have a high prevalence of vitamin D deficiency (10), which is inversely correlated to obesity, diabetes and high triglycerides (51). Therefore, this study investigated the distal and proximal muscle strength and size of the lower limbs in relation to the severity of peripheral neuropathy, IMNCT and vitamin D levels in patients with T2DM and controls. The current study shows that patients with T2DM have markedly reduced muscle strength in both proximal (knee extensors) and distal (ankle plantar flexors) muscles of the lower limb (26, 43, 48, 52). The reduction in knee
extensor strength was related to knee extensor and flexor muscle atrophy. However, the reduction in ankle plantar flexor muscle strength was not related to the reduction in muscle size although there was a trend for patients with T2DM to have smaller distal muscles compared to controls. Although, the significant increase in IMNCT in the lower limb in patients with diabetes may result in an increase of the distal muscles. Increased intramuscular fat in the distal muscles has also been associated with obesity and increased insulin resistance (46, 53, 54) and the severity of neuropathy. The current study found that reduced muscle strength in patients with T2DM was not related to small fibre neuropathy assessed using CCM but was related to the NDS. Knee extensor but not ankle flexor muscle strength was significantly reduced in patients with DSPN compared to diabetic patients without DSPN. This study suggests that neuropathy may lead to a reduction in physical activity (46) which consequently reduces the use of knee extensor muscles during walking. Nomura et al. showed that there was a negative correlation between knee extensor muscle strength and insulin resistance in diabetic patients with and without DSPN (55). Although reduced muscle strength and size and increased IMNCT were related to severe vitamin D deficiency (49, 52), this study did not show a significant correlation between these variables and muscle strength, size and IMNCT with inadequate levels of vitamin D (25(OH)D <25nmol/L) in patients with T2DM. My study supports a role for vitamin D deficiency and muscle impairment, particularly in T2DM with severe vitamin D deficiency.
7.4 Distal lower limb strength is reduced in subjects with impaired glucose tolerance and is related to increased intramuscular fat and vitamin D deficiency (chapter 5)

Detailed assessment of motor function in terms of investigation of muscle strength and size in subjects with IGT is limited. Moreover, when muscle strength and reflexes were examined clinically previously, they showed no abnormality (56). Thus, in the present study detailed quantification of lower limb muscle strength and structure in relation to IMNCT, neuropathy and vitamin D deficiency in subjects with IGT compared to controls was undertaken. The data showed that subjects with IGT had an early reduction in ankle plantar flexor muscle strength compared to control subjects, while knee extensors strength was maintained. It is in contrast with our recent findings of markedly reduced muscle strength in both distal and proximal muscles of the lower limb in patients with T2DM, which was worse in those with severe DN (57). In this study, distal muscle strength was reduced significantly whilst distal muscle volume did not differ between subjects with IGT compared to controls. A previous study found quite marked distal muscle atrophy, particularly in T2DM patients with symptomatic neuropathy (58). Although there was no significant change in proximal muscle volume in subjects with IGT, it correlated with sural nerve conduction velocity and amplitude, reflecting the severity of distal neuropathy. The severity of peripheral neuropathy is related to muscle weakness and atrophy of the distal muscles in patients with T2DM (26, 43, 44, 48, 59). An early reduction in the distal muscle strength and small fibre neuropathy in subjects with IGT (60) and T2DM (61) has been
established in the present study. The possible reason for reduced distal muscle strength was the presence of neuropathy in subjects with IGT (62, 63). In addition to significantly reduced distal muscle strength, there was a significant increase in IMNCT in the distal muscles. IMNCT has a strong relationship with obesity (64), which is consistent with our findings in subjects with IGT. Furthermore, increased IMNCT has been related to insulin resistance in subjects with IGT (65). A previous study reported that a higher infiltration of fat tissue within skeletal muscle may impair glucose consumption and fat oxidation in subjects with IGT (64) with consequent muscle weakness and motor dysfunction (66, 67). Both the reduction in muscle strength and increased IMNCT were related to a significant reduction in CNFD, which is a highly sensitive and specific measure of small fibre neuropathy in subjects with IGT (50). Increased IMNCT was also associated with a reduction in IENFD, suggesting a correlation with distal small fibre neuropathy.

Subjects with IGT and low 25(OH)D (<25nmol/l) had marked distal muscle weakness but maintained muscle strength and size in the proximal muscles of the lower limb compared to controls. In contrast with previous findings, severe vitamin D deficiency causes marked proximal muscle weakness and atrophy (68), and has also been related to increased IMNCT (49, 50). Reduced serum levels of 25(OH)D have been related to body mass in healthy subjects, which is consistent with our findings in subjects with IGT. Thus the findings show that reduced distal ankle flexor muscle strength in subjects with IGT was related to
distal small fibre neuropathy and vitamin D deficiency. Distal motor weakness may be an early feature of motor dysfunction that can occur in subjects with IGT.

7.5 Altered walking strategy and increased unsteadiness in subjects with impaired glucose tolerance and type 2 diabetes relates to small fibre neuropathy but not vitamin D deficiency (chapter 6)

Walking impairment is considered to be a late complication in patients with diabetes related to peripheral neuropathy (4, 69-72). These walking abnormalities in patients with diabetes can be found before the development of a large fibre neuropathy and has been attributed to a small fibre neuropathy (73-76), with abnormalities in walking strategy and a disturbance of balance with an increased risk of falls and severe injuries (3, 4, 69, 73, 77-79). Muscle weakness and changes in muscle structure may impair walking activity in diabetic patients without neuropathy (73). It has been shown that patients without diabetic neuropathy have slower walking speed, and shorter step length and width compared to controls (74-76). In one of our recent studies, we found that patients with T2DM with mild neuropathy had reduced lower limb muscle strength with minimal changes in the muscle structure compared to a healthy control group (57). Early small fibre neuropathy measures were established using CCM and IENFD to investigate their relationship to the biomechanical walking strategy and dynamic steadiness during walking in subjects with IGT and T2DM. Muscle
weakness, atrophy, disturbance of balance and an increased risk of falls have been associated with vitamin D deficiency during upright quiet standing and walking in otherwise healthy elderly subjects (80-82). Reduced serum levels of 25(OH)D can cause proximal muscle weakness, impaired dynamic sway during walking and hence an increased risk of falls (24, 81, 82). Thus, the natural history of alterations in walking strategy and unsteadiness in subjects with IGT and T2DM in relation to small and large fibre neuropathy and vitamin D deficiency was investigated in this study. To the best of our knowledge, there are no previous studies which have demonstrated walking and dynamic sway alterations during walking in subjects with IGT. The current study found that dynamic medio-lateral sway during walking was related to small rather than large fibre neuropathy in subjects with IGT and T2DM. Reduced ankle flexor muscle strength in subjects with IGT (83) and patients with T2DM (57) may cause walking and balance impairments in these subjects. Thus, subjects with IGT had a significant reduction in the ankle plantar flexor power but not strength during walking compared to controls. However, patients with T2DM had a significant reduction in both ankle plantar flexor strength and power during walking (71, 84, 85). Reduced cognitive function has been proposed as a possible reason for reduced walking speed in patients with T2DM (69, 73) together with increased balance instability during walking (73).

Previous studies have demonstrated that patients with DN have a slow walking speed, short step length and greater step width during walking (70, 75, 84). These alterations in walking strategy are considered to be biomechanical
compensatory strategies (80) in response to a reduced ankle strength and power during walking in patients with diabetes. In this study, reduced walking speed was shown in T2DM whilst it was maintained in subjects with IGT. However, increased step width was shown in IGT compared to controls. This may be due to a greater BMI in this group, with the need for an increase in their base of support during walking. Increased step width may lead to impairment in dynamic sway during walking which may increase the possibility of falls. Thus, a wider step during walking may be an early feature that can be seen in subjects with IGT. This greater dynamic medio-lateral sway during walking in subjects with IGT compared to controls has been found in a previous study in patients with DPN (80). The impairment of balance during walking in subjects with IGT may also be attributable to peripheral neuropathy, in particular small fibre neuropathy (72, 80, 86). Altered balance during walking in diabetic patients may occur secondary to reduced sensory and proprioceptive inputs (69, 71, 72, 78, 84). However, the current study showed no relationship between large fibre neuropathy assessed using VPT and electrophysiology with increased medio-lateral sway. In contrast, there was a significant correlation between small fibre neuropathy and medio-lateral sway in subjects with IGT.

Vitamin D deficiency plays an important role in postural unsteadiness during quiet standing, which may result in an increased risk of falls in healthy subjects (87). In this study, there was no correlation between 25(OH)D levels and walking and dynamic sway alterations between groups. It may partly reflect the reasonable levels of serum 25(OH)D, with the majority of patients not being at
deficient or severely deficient levels. It is generally agreed that vitamin D deficiency mainly affects proximal trunk muscles but not distal muscles of the lower limb (24, 87, 88). I have found that patients with T2DM with lower vitamin D (25(OH)D <25nmol/L) had significantly increased dynamic medio-lateral sway compared to diabetic patients with higher values of vitamin D.

7.6 Study limitations

This study has a number of limitations:

- The number of study participants was small.
- Neuropathy assessment and 25(OH)D concentration were derived from recent data.
- Maximal isokinetic muscle contraction was not required during assessment of muscle strength for both proximal and distal muscles using an isokinetic dynamometer (Cybex).
- Knee flexors and ankle dorsiflexor muscle strength were not assessed.
- A longer walking distance is needed during the gait analysis test.
- Participants with severe neuropathy and severe vitamin D deficiency were not included in the current study.
- Scanning the full length of each muscle using the MRI scanner could not be undertaken in one scan as the participant needed to be moved according to the MRI coil’s position.
• Actual muscle size could not be measured separately when measuring the cross sectional area as it included connective tissue and fat tissue within each muscle.

7.7 Future work

• A randomized placebo controlled trial of vitamin D in patients with diabetes and IGT with inadequate levels of vitamin D should be undertaken to investigate its effect on muscle function and structure and walking activity.
• The effect of a resistance exercise program on motor function of the lower extremities in patients with IGT and T2DM in relation to the severity of neuropathy should be investigated.
• Ascending and descending walking trials should be conducted using stairs to assess balance and risk of falls in patients with DN and vitamin D deficiency.

7.8 Conclusion

In conclusion, this thesis has shown that patients with T2DM have marked muscle weakness in both distal and proximal muscles of the lower limb which was related to the severity of DSPN but not the level of vitamin D. Proximal muscle weakness was related to proximal muscle atrophy. However, distal muscle weakness was not related to distal muscle atrophy due to increased intramuscular fat in the distal muscles in patients with T2DM and subjects with
IGT. Reduced distal muscle strength is an early deficit, which can be seen in subjects with IGT and which may be related to vitamin D deficiency and small but not large fibre neuropathy.

Reduced ankle plantar flexion strength is associated with the early abnormalities in walking strategy and balance in patients with T2DM and subjects with IGT. Walking and balance abnormalities occur early in subjects with IGT, before the development of diabetes, and become more manifest with the increasing severity of peripheral neuropathy. Early small but not large fibre neuropathy has been related to balance and walking deficits in subjects with IGT. Vitamin D deficiency per se was not related to walking impairment and disturbance of balance in subjects with IGT but was related to increased dynamic medio-lateral sway in patients with diabetes.
7.9 References


CHAPTER IX. APPENDIX 1- STUDY RELATED DOCUMENTS
8.1 PARTICIPATION INFORMATION SHEET

Study title:

“Vitamin D deficiency in relation to neuropathy and muscle function in diabetic patients”

You are being invited to take part in a research study being conducted at the University of Manchester and Manchester Metropolitan University. Before you decide whether or not to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and ask any questions you may have to the research team afterwards.

Why have you been selected?

You have been diagnosed with type 2 diabetes and vitamin D deficiency and are aged between 18-80 years, or you have been selected as part of a non-diabetic comparison group. Patients with currently on vitamin D supplements, peripheral neuropathy due to non-diabetic causes, psychiatric disorder or cognitive impairments, psychiatric or cognitive problems, foot ulcers or amputee limb, and pregnant women will be excluded from this study. Therefore, if you have any of these problems you will not take part in this study.

What is the purpose of the study?

The main objective of this study is to investigate the influence of vitamin D deficiency on motor function in patients with diabetes (with or without diabetic peripheral neuropathy).

Who is organizing this study?

This study is organized by University of Manchester, with assessments also being conducted at the Manchester Metropolitan University.

What will I have to do if I take part?
You will be asked to sign a consent form to show that you understand what is involved in taking part and we will ask you to come in for a number of measurement sessions.

The time commitment for each assessment session is indicated below. If we perform all measurements in one day it would probably take around 5 hours, so this may involve you coming in for one morning or afternoon session. It is also possible to split these assessments onto different days so that the sessions are much shorter if you prefer.

We can reimburse your travel expenses to attend the university (we will just need a public transport receipt, details of car mileage etc.).

During the first laboratory visit we will ask about your medical history, test nerve damage (peripheral neuropathy) in your feet. Tests for peripheral neuropathy will involve pressing on different areas of your feet and also placing a vibrating device on your feet to see if you can detect these sensations.

This study involves a number of separate tests/assessments:

1. **Strength assessment**: We will measure the strength of your calf and front thigh muscles using a strength-measuring device. To assess front thigh strength you will be seated and asked to push as hard as you can by trying to straighten your leg against a fixed pad. Your leg will not move but we will assess the strength produced. You will be asked to do this a few times with your leg in slightly different positions. To assess calf strength you will lie on your front, with your foot secured into a footplate. You will be asked to push down with your toes and foot against the fixed footplate as hard as you can. The footplate will not move, but we will measure the strength. (This assessment will take around one hour to be completed)

2. **MRI scanning**: This test does not involve any radiation and is perfectly safe for the vast majority of people. This scanner does have a very large permanent magnet and therefore for your safety if you have one of the following criteria you will be excluded from undergoing this particular test (MRI scanner); women who are or may be pregnant, Ferromagnetic foreign bodies, Cardiac pacemakers/cardioverter defibrillators, Cochlear implants, Intrauterine devices and Implanted drug infusion pumps.

We will measure the size of your calf and thigh muscles using a 0.25 Telsa MRI scanner device. To assess right leg muscles size (thigh and calf) you will be lying on your back in a comfortable position. Oil-filled capsules will be used as markers placed onto your skin along your leg and thigh and fixed using adhesive tape. You will be asked to be relaxed during the scan without any movement. (This test will take approximately one hour to be completed).

3. **Gait Analysis**: To assess your walking pattern and body sway, you should be able to walk independently without using any assistive device for a short distance (around 10 meters). You will be asked to wear specialist diabetic footwear and suitable clothing (t-shirt and short) that we will provide you with to help us to put reflective markers onto
specific parts of your whole body. These markers will then be seen and tracked by the cameras in our laboratory to assess how you walk. You will simply be asked to walk as you do naturally, up and down a 10-meter walkway in our laboratory and to repeat this a number of times. We can stop and take a break whenever you like and we can take as much time in between repeated walks and you need. (This test should last approximately one hour).

4. **DEXA:** With this test we will scan your bones to assess their density. You will be lying down and the test will take approximately 20 minutes. This test does involve a small dose of radiation, but this is relatively small compared to an X-Ray and is equivalent to the radiation dose that you would get by being on a flight from Manchester to Paris.

**What are the potential risks or discomfort?**

There are very few risks may be including in this study, such as pain during leg movement and hypersensitivity to light touch particularly in patients with diabetic peripheral neuropathy. There are potential risks for some people for the scanning techniques of MRI and DEXA, but we will make sure that these tests are safe for you before we undertake them. If we are in any doubt about your stability, you will not undergo these scans.

**Are there any possible benefits?**

You will receive feedback on how you walk and your level of leg muscle strength and bone density.

**Do I have to take part?**

No, taking part is **entirely voluntary.** If you would prefer not to take part you do not have to give a reason. If you do take part but later change your mind you can withdraw from the study at any time.

**GP Letter.**

If you are a person with diabetes and decide to take part, a letter will be sent with your consent to your GP to inform him/her of your participation in the study. If you part of the control group and we identify that you may have peripheral neuropathy or a particularly high level of blood glucose, with your consent we will notify your GP, who may then suggest following an appropriate course of action with you.

**What if I have any Concerns?**

If you have a concern about any aspect of this study you should ask to speak to the researchers who will do their best to answer your questions (Prof. Malik: 0161 2751196, or Monirah Almurdhi: 07539343936).

In the event that something does go wrong and you are harmed during the research and this is due to someone’s negligence then you may have grounds for a legal action for compensation against the Central Manchester NHS Hospitals Foundation Trust (+44...
(0)161 276 1234), but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate).

**Medical Records.**

If you are a person with diabetes, existing members of the clinical care team at the Central Manchester NHS Hospitals Foundation Trust may look at relevant sections of your medical notes and data. All information will be kept confidential.

**Storage and Disposal of Study Data**

All research data will be held in secure storage at the University of Manchester. All participant data will be anonymous and only identified by a unique number.

**What do I do now?**

Thank you very much for considering taking part in our research. Please discuss this information with your family, friends or GP if you wish.

If you would like to obtain any further information about this research project please contact a member of the research team or the research nurse by e-mail or telephone.

They will then answer any questions you might have and if you are interested will arrange a convenient appointment for you to attend for your initial visit.


<Researcher’s Name, at the University of Manchester>

<Researcher’s email address>

<Researcher’s telephone number>
Participant Code: ……………………

8.2 PATIENT CONSENT FORM

Title of Project: Vitamin D deficiency in relation to neuropathy and muscle function in diabetic patients

Chief Investigator: Prof. Rayaz Malik  Principal Investigator: Monirah Almurdhi

I confirm that I have read and understand the information sheet dated 17/01/2013 (version1) for the above study and have had the opportunity to ask questions.

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

I agree to take part in the above study.

I agree to my GP being informed of my participation in the study.

I agree to video recording of me being taken while walking.

I agree to members of the research team at the Diabetes Centre looking at my medical notes.
I agree to my anonymised information being exported to members of the research team at the University of Manchester.

I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

____________________________________    ________________________________    _______
Name of Participant                        Signature                           Date

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Name of Person taking consent ________________________________
### 8.3 ISOKINETIC DYNAMOMETER (CYBEX) SHEET

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8.4 SCREENING & CONSENT FORM FOR MAGNETIC RESONANCE IMAGING (MRI)

It is important that you read and understand the questions below since we cannot scan you until they are all answered. Please do not sign the form unless you are happy with the information you have given.

Delete as appropriate

1. Have you ever had an MRI scan before?
   Yes/no If so, where?...........................................

2. Do you have a cardiac pacemaker or have you ever had heart surgery?
   Yes/no

3. Had you ever had any operations on your heart, head, neck or back?
   Yes/no
   Please give details..........................................................

4. Have you ever had any other operations?
   Please give details................................................................

5. Have any of these operations involved the insertion of metal pins/plates/implants?
   Yes/no
   Please give details................................................................

6. Is there any possibility that you may have metal from a previous injury, e.g.
   a) have you ever had metallic fragments in your eyes?
      Yes/no
   b) have you ever had a shrapnel or bullet injury?
      Yes/no

7. Have you ever suffered from epilepsy?
   Yes/no

8. Have you removed the following – jewellery, hair clips, keys, watches, hearing aids,
   Yes/no coins, spectacles, false teeth, other metal objects, credit cards, memory sticks?

9. Do you have any tattoos?
   Yes/no
FEMALES ONLY

10. Is there a possibility that you may be pregnant?
   Yes/no

11. Do you have a contraceptive diaphragm in situ?
   Yes/no

PARTICIPANT SIGNATURE…………………………………………… DATE………………………….

MRI OPERATIVE………………………………………………………..  DATE………………….
# 8.5 GAIT ASSESSMENT SHEET

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