The impact of diabetic neuropathy on respiratory and peripheral muscle strength in patients with type 2 diabetes mellitus

Birgit Van Eetvelde

The cover shows a detail of an embroidery by Catharina, my beloved sister

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The impact of diabetic neuropathy on respiratory and peripheral muscle strength in patients with type 2 diabetes mellitus

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Tantum modo incepto opus est, cetera res expediet.

-Gaius Sallustius Crispus (86-35 AC)-

De coniuratione Catilinae

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LIST OF ABBREVIATIONS

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ADA	American Diabetes Association
ADL	Activities of Daily Living
AKT1	Serine-threonine protein kinase AKT1
ANCOVA	univariate analysis of covariance
ANOVA	univariate analysis of variance
BIA	Bioelectrical Impedance Analysis
BMI	Body Mass Index
C	healthy Controls (without neuropathy) (*)
Ca^{2+}	Chemical notation for Calcium (cation)
cdNP	clinically diagnosed Neuropathy (*)
COPD	Chronic Obstructive Pulmonary Disease
CRT	Chair Rising Test (**)
CST	Chair Stand Test (*)
cm H_2O	centimeter water
°C	degrees Celsius
° s^{-1}	degrees per second
D	patients with type 2 diabetes mellitus (*)
D-	patients without clinically diagnosed Neuropathy (*)
D+	patients with clinically diagnosed Neuropathy (*)
DF	Dorsiflexion
DM	Diabetes Mellitus
dNP	diabetic Neuropathy (**)
dNP-	Patients without diabetic neuropathy (**)
dNP+	patients with diabetic neuropathy (**)
dNPs	(Patients with) sensory diabetic neuropathy (**)
dNPsm	(Patients with) sensorimotor diabetic neuropathy (**)
DNS score	Diabetic Neuropathy Symptom score
DXA	Dual-energy X-ray Absorptiometry
EASD	European Association for the Study of Diabetes
ENMG	Electroneuromyography

FM^{tot}	Total fat mass
GLUT4	Glucose transporter type 4
GOLD	Global Initiative for Chronic Obstructive Lung Diseases
HbA1c	Haemoglobin A1c or glycated haemoglobin
HC	Healthy Controls (without neuropathy) (**)
HDL-C	High-density lipoprotein cholesterol
HGS	Handgrip Strength
hs-CRP	high sensitive C-reactive protein
IK	Isokinetic (dynamic)
IM	Isometric (static)
IMT	Inspiratory muscle training
IR	Insulin Resistance
J	Joule
K^+	Chemical notation for Potassium (cation)
kg	kilogram
L/m	Liter per minute
LBM	Lean Body Mass
LBM^{tot}	total Lean Body Mass
LBM^{arm}	Lean Body Mass of the dominant arm
LBM^{leg}	Lean Body Mass of the dominant leg
LDL-C	Low-density lipoprotein cholesterol
m	meter
M.	Musculus / muscle
mg/cm^3	milligram per square centimetre (muscle density)
mmHg	millimeter of mercury
MNSI	Michigan Neuropathy Screening Instrument
MRI	Magnetic Resonance Imaging
m/s	meter per second
µV	microvolt
mV	millivolt
N	Newton
N.	Nervus / nerve

Na^+	Chemical notation for Sodium (cation)
NCS	Nerve Conduction Studies
Nm	Newton-meter
NP	Neuropathy
NSS	Neuropathy Symptom Score
% 1RM	percentage of one Repetition Maximum
PEF	Peak Expiratory Flow
PE_{max}	Maximal (static) Expiratory Pressure
PI_{max}	Maximal (static) Inspiratory Pressure
PF	Plantar Flexion (ankle) / Palmar Flexion (wrist)
PKC	Protein kinase C
pQCT	Peripheral Quantitative Computed Tomography
PT	Peak torque
QoL	Quality of Life
Reps	repetitions
RM	Repetition Maximum
RoM	Range of (joint) Motion
ROS	Reactive Oxygen Species
s2LJ test	Single two leg jump test
SD	Standard deviation
SPPB	Short Physical Performance Battery
SPSS	Statistical Package for Social Sciences
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TNF-α	Tumor necrosis factor-alpha
VPT	Vibration Perception Threshold
W	Watt
W/kg	Watt per kilogram body weight

(*) abbreviations used in original research 1

(**) abbreviations used in original research 2 and 3

PART I

INTRODUCTION, AIMS AND MAIN RESEARCH QUESTIONS

PART I: INTRODUCTION, AIMS AND MAIN RESEARCH QUESTIONS

1. Type 2 diabetes mellitus

1.1. Definition and prevalence

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease characterized by elevated levels of fasting blood glucose (i.e. hyperglycemia), resulting from impaired insulin secretion, resistance to insulin action, and excessive or inappropriate glucagon secretion [1]. The prevalence of diabetes, in particular T2DM (accounting for around 90% of all diabetes cases), has nowadays reached proportions of a pandemic. According to data published in the latest Diabetes Atlas of the International Diabetes Federation, 2017, the disease prevalence is already 425 million people worldwide. This number is estimated to rise to 629 million by the year of 2045, driven by a complex interplay of genetic, social, demographic and environmental factors [2].

1.2. Physiology of glucose homeostasis

In the 10-12 hours overnight fast, known as the post-absorptive state, approximately 50% of the total body glucose disposal is utilized by the brain, and another 25% in the splanchnic (hepatic and gastrointestinal) area, both being insulin independent. The remaining 25% of glucose metabolism primarily takes place in insulin-dependent muscle tissue. The liver produces approximately 85% of all endogenous glucose. The remaining amount is produced by the kidneys. Glucose ingestion triggers this balance between endogenous glucose production and tissue glucose uptake. Plasma glucose concentration is increased (hyperglycemia) and stimulates insulin release from the pancreatic β-cells in the islets of Langerhans, which results in hyperinsulinemia. Simultaneously with insulin secretion into the portal vein, the release of glucagon from the pancreatic α-cells is inhibited. Consequently, due to this new hormonal ratio, endogenous glucose production is suppressed and glucose uptake by splanchnic and peripheral muscle tissues is stimulated. The maintenance of this whole-body glucose homeostasis is dependent upon a normal insulin secretory response by the pancreatic β-cells and normal tissue sensitivity to augment glucose uptake [3].

It is mandatory to know that insulin is an endocrine peptide hormone with major **direct** glucose-lowering effects on three insulin target cell types: myocytes (skeletal muscle tissue), hepatocytes (liver tissue) and white adipocytes (adipose tissue). The first direct

insulin-signaling pathway includes the insulin receptor and the activation of its direct substrates, initiating the insulin response at the plasma membrane of target cells [4-6]. Secondly, this activation triggers intracellular mechanisms, including different protein kinases (PKC, AKT1, …), finally increasing translocation of Glucose transporter type 4 (GLUT4) primarily to the cell membrane of myocytes and adipocytes, and GLUT2 mainly to the cell membrane of hepatocytes [3, 7]; thereby promoting glucose uptake, storage and utilization. The third direct insulin action is the immediate suppression of hepatic glucose production.

Insulin also exerts important **indirect** effects, such as the suppression of glucagon secretion in the pancreatic α-cells. This is an elegant and tightly controlled reciprocal paracrine regulatory system in order to favour either insulin or glucagon secretion, but not both, in any given metabolic state [5].

1.3. Pathophysiology of insulin resistance and type 2 diabetes mellitus

One of the earliest characteristics in the pathogenesis of T2DM is insulin resistance (IR). The disease is fully diagnosed when IR is accompanied by impaired insulin secretion, and when chronically disturbed glucose homeostasis is established in fasting blood conditions. Concomitant to the insulin deficit, there is an excessive release of plasma glucagon (particularly in the post-prandial period). This imbalanced insulin-glucagon ratio is associated with increased lipid infiltration in the skeletal muscles and is also linked to functional impairment of the cellular mitochondria, resulting in an overproduction of reactive oxygen species (ROS). In normal circumstances, ROS are formed as a natural by-product of normal mitochondrial oxygen metabolism and these low ROS concentrations play an important role in cellular homeostasis. In these conditions, there is a balance between ROS formation and antioxidants. However, in patients with T2DM, ROS levels increase dramatically and the antioxidant defences become insufficient, resulting in oxidative stress which may lead to both cell membrane and cell DNA damage, and eventually to cell apoptosis in the end. This cell damage will lead to increased inflammatory responses with increased pro-inflammatory cytokines (e.g. interleukins, TNF-α, hs-CRP, …). ROS, as well as these cytokines, have a negative impact on the insulin-signaling pathway in several tissues leading to decreased GLUT4-translocation into the cell membrane. This results in a reduced uptake of glucose and an increased glucose blood level. Glucose uptake in patients with T2DM uniformly has been found to be decreased in

adipocytes and myocytes. In both cell types, the ability of insulin to elicit a normal translocation and to activate the GLUT4 transporter after its insertion into the cell membrane is impaired [3, 8, 9].

During the initial phase of the pathogenesis of T2DM, pancreatic β-cells compensate for the insulin resistance by upregulating the secretion of insulin. This β-cell compensation period is followed by β-cell failure resulting from either inadequate expansion of β-cell mass or failure of the existing β-cell mass to respond to glucose, both due to a defect in insulin-signaling [4-6]. Several mechanisms (e.g. the activation of endoplasmic reticulum stress, the generation of ROS, and the induction of pro-inflammatory cytokines in pancreatic islets) are thought to adversely affect β-cells by reducing their capacity to proliferate, impairing insulin secretion, decreasing insulin gene expression, and ultimately promoting uncontrolled β-cell death.

1.4. Diagnostic criteria and standard treatment

The gold standard diagnostic criteria, based on the American Diabetes Association (ADA) guidelines, include (i) an HbA1c level (i.e. glycated Haemoglobin or Haemoglobin A1c level) of 6.5% or higher, based on a laboratory method that is certified by the National Glycohaemoglobin Standardization Program and standardized or traceable to the Diabetes Control and Complications Trial reference assay, or (ii) a fasting (no caloric intake for at least 8 hours) plasma glucose level of 126 mg/dL or higher, or (iii) a two-hour plasma glucose level of 200 mg/dL or higher during a 75-g oral glucose tolerance test, or (iv) a random plasma glucose of 200 mg/dL or higher in a patient with classic symptoms of hyperglycemia, such as polyuria, polydipsia, polyphagia, and weight loss, or hyperglycemic crisis [10].
However, the American Association of Clinical Endocrinologists recommend that HbA1c has to be considered as an additional optional diagnostic criterion rather than a primary criterion for the diagnosis of diabetes [11].

A standard treatment statement for patients with diagnosed T2DM has been described by ADA in combination with the European Association for the Study of Diabetes (EASD) recommendations and contains seven key points:

1. Individualized glycemic targets and glucose-lowering therapies.
2. Diet, physical activity/exercises, and diabetes education as the cornerstone of the treatment program. This fundamental implementation should counter the decline in

skeletal muscle mass and strength in patients with T2DM as key elements to sustain daily functioning, walking speed, and physical performance in order to maintain or even enhance quality of life (QoL).

3. Use of metformin, the only biguanide in clinical use, as the optimal first-line drug unless contraindicated.
4. After metformin, the use of one or two additional oral or injectable agents, with a goal of minimizing adverse effects if possible.
5. Ultimately, insulin therapy alone or with other agents if needed to maintain blood glucose control.
6. Where possible, all treatment decisions should involve the patient, with a focus on patient preferences, needs, and values.
7. A major focus on comprehensive cardiovascular risk reduction [12].

Furthermore, some approaches in the **prevention of diabetic comorbidities** have been formulated, such as a check-up on the HbA1c level every 3-6 months, yearly dilated eye examinations, annual microalbumin checks, foot examinations at each visit, blood pressure measurements <130/80 mmHg (even lower in diabetic nephropathy), and statin therapy in order to reduce low-density lipoprotein cholesterol (LDL-C) [12].

1.5. Acute and chronic complications

Although T2DM is particularly associated with chronic disorders, some acute complications may occur, such as hypoglycemia, represented by fasting plasma glucose levels beneath 70 mg/dL, and hyperglycemia, when fasting plasma glucose level exceeds 126 mg/dL. However, these important acute complications will not be further discussed, as hypo- and hyperglycemia do not relate to our field of research interest as such.

Patients with T2DM are at high risk to develop vascular complications, as detrimental effects of consistent hyperglycemia, resulting in high morbidity and mortality. These diabetic vascular complications are classified as either macrovascular (cardiovascular, cerebrovascular, and peripheral artery disease) or microvascular (retinopathy, nephropathy, and neuropathy) [13-16]. The 'unified hypothesis' proposes that overproduction of ROS is triggered by the increased oxidation of free fatty acids, induced by insulin resistance, and by intracellular hyperglycemia in the development of respectively macrovascular (prevalence of 30%) and microvascular (prevalence of 50%) complications [17, 18].

As diabetic neuropathy (dNP) is the most frequent microvascular complication of T2DM, and as motor dysfunction is a late and severe manifestation of dNP, with possible impaired gait and poor balance and coordination [19, 20], this complication in particular is of our interest and has to be fully comprehended for physiotherapy purposes.

Hence, by definition, neuropathy affects the long peripheral autonomic and/or somatic nerves in approximately 10-15% of all people aged more than 40, in which diabetes is the most common cause. The autonomic nerve system regulates involuntary physiological processes, such as blood pressure, digestion, and respiratory rate [21]. The somatic nervous system controls voluntary muscles within the body and the process of voluntary reflex arcs, which includes sensory and motor nerves [22]. Consequently, neuropathy can be autonomic and/or sensory or sensorimotor [23].

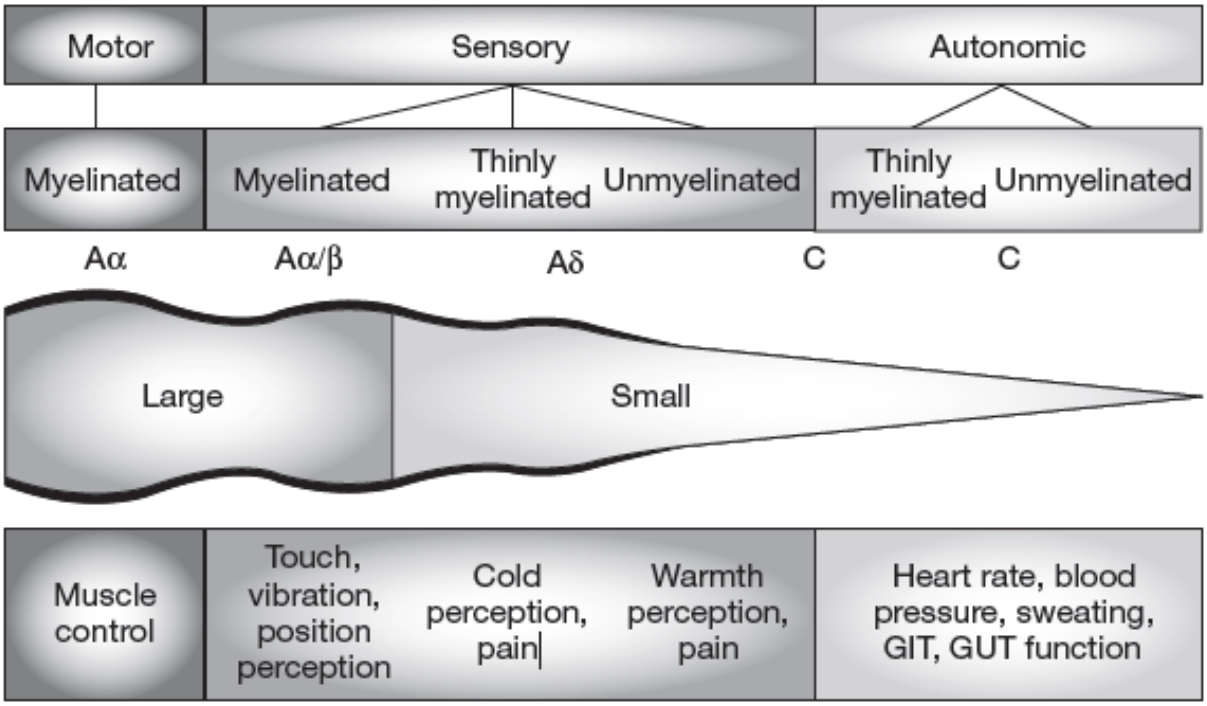


Figure 1 A simplified view of the peripheral nervous system. Clinical presentation of small and large fiber neuropathies. Aα fibers are large myelinated fibers that mediate motor functions and muscle control. Aα/β fibers are large myelinated fibers that mediate perception of touch, vibration and position sense. Aδ fibers are small myelinated fibers, transmitting pain stimuli and cold perception. C fibers can be myelinated or unmyelinated and have both sensory (warmth perception and pain) and autonomic neurotransmission (blood pressure and heart rate regulation, sweating, etc.) [24].

DNP and other neuropathies predominantly affect small myelinated and unmyelinated sensory fibers that convey pain and temperature sensation (see Figure 1) [24]. Degeneration in these small fiber neuropathies involves the most distal portions of these nerve fibers that are found in different organs and tissues (somatic fibers). The pathological changes of most peripheral neuropathies are axonal degeneration,

segmental demyelination or a combination of both [25]. Sensory and sensorimotor dNP affect the distal peripheral nervous system in the lower limbs inducing peripheral skeletal muscle weakness, while the proximal muscles of the legs and upper limbs are often preserved unless the diabetic neuropathy is long-standing for approximately 25-30 years [26]. In the early stages of dNP, patients often present with predominant involvement of the sensory nervous system (sensory symptoms) without detectable clinical motor impairment. After years of dNP exposure and progression, clinical impairment in motor function arises, with loss of motor axons and/or units in association with muscle weakness [27, 28]. This long-term detrimental complication (i.e. the evolution from sensory dNP (dNPs) to sensorimotor dNP (dNPsm) with atrophy of the skeletal muscle in the end) affects up to 50% of people with T2DM [26, 27, 29].

This accelerated muscular atrophy in patients with T2DM results in progressive loss of muscle strength and may cause severe motor dysfunction, often leading to poor walking performance, balance and coordination. In addition, these manifestations may increase their risk of falling [19].

2. Diabetic neuropathy

2.1. Current screening and diagnosing

The significant morbidity and mortality associated with dNP stimulated the development of methods to screen, diagnose, and assess the given condition. Generally, after the initial anamnesis of the diabetic person, a clinical neurological examination is performed [30]. This examination usually consists of a variety of assessments, e.g. checking on skin vibration, testing with monofilaments, Achilles tendon reflex, visual control of the appearance of both feet, some specific dNP questionnaires, etc [23]. However, these clinical neurological examinations are rather variable (i.e. more heterogeneous) and definitely have a less accurate outcome compared to the electroneuromyography (ENMG) screening for type and severity of dNP. ENMG, by means of sensory and motor nerve conduction studies (NCS) of large myelinated upper and lower limb nerves, have been considered the gold standard for assessing neural (dys)function and, consequently, for diagnosing neuropathy. This technique is assumed to be superior to all other assessment tools of neuropathy [26].

For that matter, caution is recommended when interpreting research results based on unconfirmed, clinically diagnosed dNP (i.e. exclusively by means of clinical neurological examination) in comparison with those based on ENMG diagnosed dNP.

2.1.1. Clinical neurological examination

In our studies, **clinical neurological examination** was performed in each study participant and comprised three parts: measurements of Vibration Perception Threshold (VPT), the Michigan Neuropathy Screening Instrument (MNSI) and the Diabetic Neuropathy Symptom (DNS) score.

VPT, usually determined by means of a Bio-Thesiometer® or a Neurothesiometer® [31] on the left and right medial malleolus and on the distal plantar surface of the big toes, is considered a valid and reliable measurement of the peripheral large-fiber sensory nerve function [32, 33]. The required level of skills for this test consists of a minimal training and the implementation of it only takes 5-10 minutes per person [23]. In some pioneering studies (1984 and 1991) the threshold was defined as the lowest recorded voltage of three readings from the moment the subjects indicated a sense of vibration, at each of the four spots [34, 35]. Subsequently, Wiles et al., 1991, decided to use four percentile rank charts of VPT variation, as they proved that the threshold at which vibration becomes perceptible is dependent of age, gender and location. Then, a normality cut-off on the 95th percentile was applied in order to decide whether vibration perception was within the normal range [34]. In our studies the same methods were used and the dNP criterion was determined as positive if one of the readings (big toe and the medial malleolus, both left and right) was above the 95th percentile.

MNSI is a composite measure tool for sensory and motor nerve symptoms and the person's peripheral vascular status [23], consisting of a personal 15-items questionnaire and a clinical neurological examination, starting with a thorough inspection of both feet. Subsequently, the presence of ulceration on both feet is checked, followed by an evocation of the Achilles tendon reflexes. At last bilateral vibration perception measurements were performed using a 128Hz tuning fork and the tactile sensation was examined by means of a test with a 10g monofilament [36-38].

The DNS score is a validated 4-point yes/no questionnaire with a high predictive value in the screening for dNP when patients score ≥1/4. Meijer et al., 2002, compared the validity, predictive value and reproducibility of the DNS with the Neuropathy Symptom Score (NSS). They found a high correlation between NSS and DNS score (Spearman's ρ=0.88) and concluded that the DNS is a fast, easy, reproducible (Cohen weighted κ 0.78-0.95) and valid assessment tool to screen for diabetic polyneuropathy [39]. Consequently, the above-mentioned cut-off was withheld as positive in our own trials.

2.1.2. Electroneuromyography

Sensory and motor NCS by means of ENMG, performed by board-certified specialists, are used to classify diabetic patients to target dNP subgroups (dNP-, dNPs, and dNPsm) in a standardised and validated way. These electrophysiological studies are, according to an American consensus declaration in 2005, sensitive, specific and validated measures for the presence and severity of dNP, and provide a high level of specificity for the diagnosis [40]. NCS is considered to be the gold standard for the diagnosis of dNPs or dNPsm [26]. Board-certified professionals can even make a distinction between symmetric or asymmetric, and axonal or demyelinated dNP.

In the vast majority of the T2DM studies, the diagnosis of dNP has been consistently confirmed using NCS, which measure the motor and sensory nerve action potentials and conduction velocities of well-defined large myelinated nerves in upper and lower limbs. The motor NCS are usually performed by stimulation of the N. Peroneus communis, N. Tibialis and the N. Ulnaris, the sensory NCS by stimulation of the N. Suralis and the N. Radialis. Furthermore, all sensory measurements have to take skin temperature of hand and foot into account, and has to be maintained at a minimum of 30°C [41, 42].

2.2. The impact on daily living and physical functioning

Physical inactivity or a decreased physical activity level is a part of the underlying mechanisms of age-related loss of muscle strength and mass, and therefore physical activity can be seen as an important factor to reverse or modify the development of this condition. There is no doubt that appropriate exercise represents the most important approach in the prevention and treatment of muscle weakness. The older population benefits from enhanced physical activity, which lowers all-cause mortality and preserves functional independence [43]. Additionally, weight losses of 5-10% have been associated with significant improvements in cardiovascular disease risk factors when checking on HbA1c, blood pressure, HDL-C, and plasma triglycerides in patients with T2DM, with even more reduction when the patient loses up to 10-15% of weight [44, 45]. However, every single patient should be aware that two and two equals five when combining dietary modifications with increased physical activity. In patients with T2DM, research showed that aerobic exercise and resistance training improves insulin sensitivity and glycemia markedly. When incorporating combined aerobic and resistance training, an even more optimal lowering of HbA1c levels and glycemia will

be achieved, besides decreased cardiovascular risk factors, such as waist circumference, systolic blood pressure, and circulating triglycerides [46-48].

In view of a targeted resistance training it is obviously important to specifically focus on eventual decreased or changed specific measures of strength. This mandatory focus on specific changes due to the disease as such may be additionally impeded and challenged by dNP as this may further inflict the strength measures. However, the additional impact of dNP on top of the T2DM disease itself on the different muscle strength parameters is still rather unexplored. For this matter and from this point of view, more in-depth research between different diabetic groups (patients without dNP, with sensory and with sensorimotor dNP) could reveal mandatory information on the particular decrease in muscle strength between these groups and, consequently, on the implementation of the best training program for each group. Actually, it is known that the disease itself affects these parameters and, additionally it can be presumed that worsening data are yielded when dNP is present with even an accelerating effect in the dNPsm group.

Furthermore, the impact of dNP on the patients' QoL is tremendous [24]. On one hand, T2DM and sensory/sensorimotor dNP have been correlated to metabolic and inflammatory changes, accelerating the age-related decreases in skeletal muscle strength and muscle mass, and, therefore, potentially contributing to the development of disability in daily life activities. Consequently, T2DM and dNP can lead to an impaired balance and gait, which in turn increases risk of falls and loss of independence [49-51]. As dNP is amongst the most frequent complications of T2DM, this condition often is the primum movens to increased functional impairment, morbidity, mortality, and economic burden (mild to severe foot ulceration, gangrene, limb loss, …). On the other hand, cardiovascular autonomic dNP causes increased morbidity and mortality to diabetic patients with exercise intolerance and increased cardiovascular risk as major clinical manifestations. The limited exercise response experienced by these patients, in turn, generates low levels of physical activity and poor cardiorespiratory fitness. This may result from reduced responses in heart rate, blood pressure, and cardiac output [52], as well as decreased maximal strength and muscle endurance of the inspiratory muscles [52-54].

3. Peripheral skeletal muscle strength

The peripheral skeletal muscles at interest for this dissertation are located at knee, ankle, elbow and hand, and are all innervated by large myelinated Aα fibers that mediate motor functions and muscle control. Relying on previous study protocols, our research focussed on the analytical assessment of skeletal lower limb muscle strength, analyzing knee extensor muscles (vastus medialis, intermedius, lateralis, and rectus femoris) and flexors (semi-membranosus, biceps femoris, and semitendinosus) separately, and ankle plantar flexor muscles (soleus, and the medial and lateral head of the gastrocnemius) and dorsiflexors (tibialis anterior and extensor digitorum longus) [20, 27-29, 49, 55-73]. Concerning the upper limbs, full focus was laid on elbow extensors (the lateral, medial and long head of the triceps), flexors (biceps brachii, brachialis and coracobrachialis), and handgrip musculature (flexor digitorum superficialis and profundum), again in an analytical way [55-60, 74-81]. With respect to reflections regarding the same previous research, additional attention was given to functional tests corresponding more to the older person's/patient's behaviour, ability on daily life activities, QoL, etc. …

3.1. Definitions and assessments

Physical function (which is a synonym for physical fitness) is defined as one's ability to carry out activities that require physical actions, ranging from self-care (activities of daily living (ADL)) to more complex activities that require a combination of skills, often with a social component or within a social context [82]. Physical function/fitness as such is a multidimensional concept with four health-related components: **morphological**, **muscular**, motor and cardiorespiratory (Table 1) [83].

Table 1 Components and factors of health-related physical function/fitness [83]

Component	Factors
Morphological	Body Mass Index
	Body composition
	Abdominal visceral fat
	Flexibility
Muscular	Maximal muscle strength
	Muscle endurance
	Explosive strength/power
Motor skills	Balance
	Speed
Cardiorespiratory	Submaximal exercise capacity
	Maximal aerobic power

Hence, the **morphological component** plays an important role in the recruitment of participants in view of the close association with obesity, T2DM, dyslipidemia, hypertension and increased cardiovascular risk. In this dissertation, whole and segmental body composition was taken into account as an important influencing factor, certainly for the **muscular component**, which is the focus of the original research.

By using a helicopter view (top down), **physical function** as 'general concept' consists of two major domains: **physical performance** and **physical activity**. As physical performance is 'ability' and physical activity is 'behaviour', it could be assumed that they are related but also represent separate domains of physical function (Figure 2) [82].

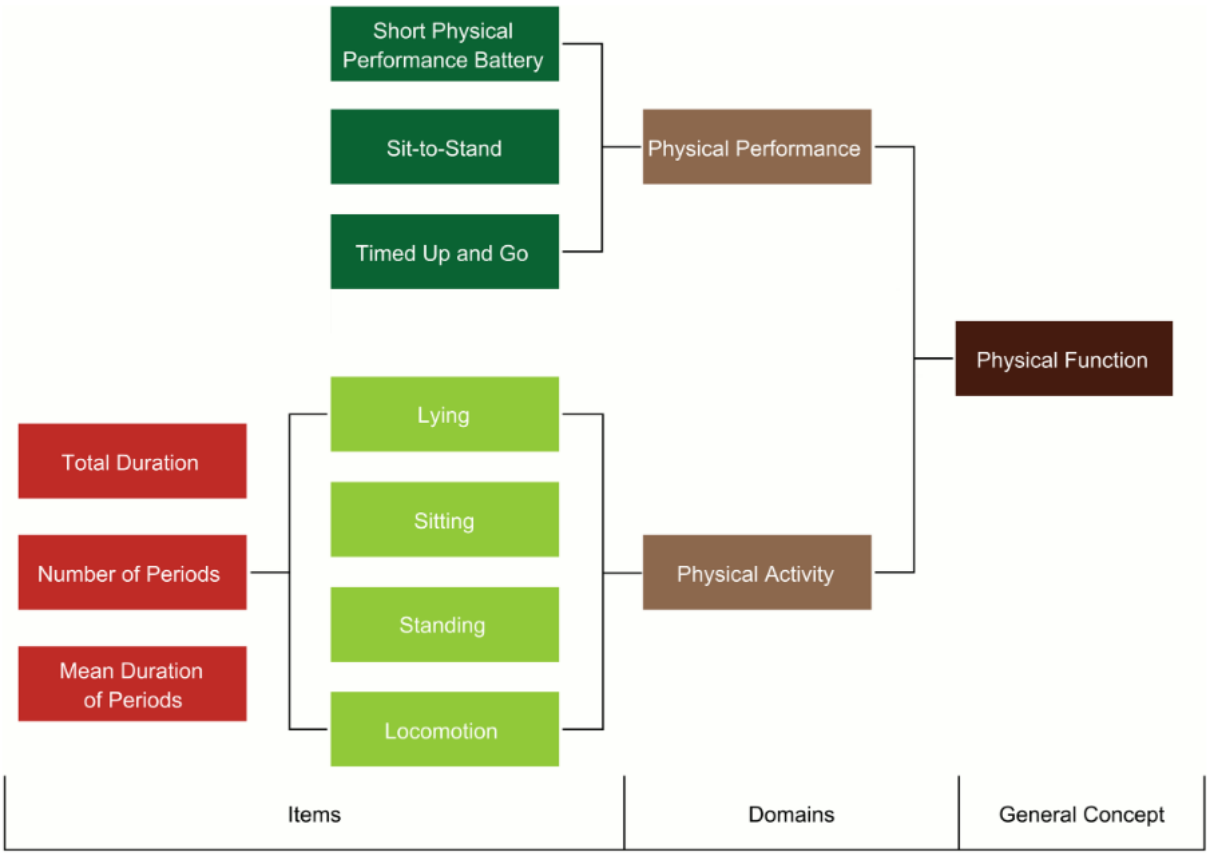


Figure 2 Mobility measures presented in a framework with physical performance and physical activity as domains of physical function. Activity classes are determined and for all types of physical activity total duration, number of periods and mean duration of periods are calculated [82].

Physical performance is the ability to perform physical activities. Consequently, poor physical performance implies that one cannot reach the physical demands of daily living. As typical outcome measures (such as the time to perform a supervised and standardized task, the Short Physical Performance Battery test, the timed Sit-to-Stand test, and the Timed Up and Go test) are straightforward to determine a clear objective, these tests are widely applied [82].

Physical activity is defined as any bodily movement produced by skeletal muscle contractions and encompasses a complete spectrum of activities, from very low levels of energy expenditure to maximal exertion. Physical activity includes not only exercise (such as walking, running, and cycling), but also certain work-related activities (e.g. lifting), household activities (e.g. cleaning), leisure activities (e.g. gardening), and active play or recreation (such as competitive sport, working out in a gym and dancing). Self-reporting questionnaires and activity diaries were the most commonly used methods, albeit subjective, to measure physical activity [82-84]. Nowadays, objective analysis methods (ambulatory movement registration techniques, such as accelero- and pedometers, activpal inclinometers, and activity- and GPS-trackers) have been

developed to differentiate activities like lying, sitting, standing and locomotion which allow objective assessment of intensity, frequency and duration of physical activities [82].

Minimal levels of physical function/fitness are needed to perform those daily life activities in order to maintain functional independence in lower and upper limbs as one ages, and in order to be able to participate in active leisure-time activities without strains, stress or fatigue. Therefore, appropriate attention for the maintenance of skeletal muscle strength parameters in lower and upper limbs of the ageing population, and in particular the ageing diabetic patient, is mandatory [84-86].

Maximal muscle strength is defined as the ability of a voluntary muscle(group) to generate maximal contractile force against an external resistance in one single contraction [84]. Maximal voluntary muscle strength in the upper and lower limbs can be measured isometrically (IM) and/or isokinetically (IK), the latter being a closer reflection of muscle function in everyday activity. Most studies describe maximal muscle strength as peak torque (Newton-meter; Nm), being the magnitude of force generation, and the work rate as power (Watt; W), i.e. work done per unit time [87].

Muscle endurance is the ability of a muscle(group) to exert submaximal force during an extended period [84]. Total work represents the work produced throughout the test, i.e. the muscle's capability to maintain torque throughout the test bout. Work in first third and last third are the efforts produced during the first and last thirds of the test duration, respectively. These are useful measures to determine work-fatigue, which is the percent decrease in torque output between the first third and the last third of work, divided into the first third of work in the test. This is a valuable parameter in documenting progress during endurance training in order to detect the fatigability throughout the test bout [88]. **For both strength parameters**, it has to be mentioned that the generated force is highly dependent on the velocity of movement (dynamic (IK) versus static (IM) measurements). Isokinetic contraction is the maximal generated force of a muscle(group) at a constant velocity (e.g. 60° s^{-1} or 180° s^{-1}) throughout the entire range of joint motion (RoM) [84].

Explosive muscle strength or power is also a dynamic strength parameter. It represents the characteristics of the muscle-nerve system to conquer any resistance with the highest generated contraction velocity. Hence, the difference between maximal muscle strength and explosive strength relies on the time to develop maximal

strength [84]. Explosive body strength by means of total muscle power can be easily measured by counter-movement jumps (e.g. single two leg jump and multiple hop test) and a chair rising test. Total muscle power (W) is the key outcome parameter since it is defined as the ability to generate as much force as possible and as quickly as possible. This value is calculated as the product of muscle force (Newton; N) and velocity (m/s), and is often corrected for body weight (W/kg). Hence, altered neural or muscular ability affecting either factor (force or velocity) will contribute to declines in power and potentially physical functioning. Therefore, total muscle power is a valuable measure for identifying age-related physical impairments and strongly correlates with physical capability, mobility, the risk of falling, and sarcopenia [89-92].

3.2. State of the art in patients with diabetic neuropathy

3.2.1. Maximal muscle strength

Muscular atrophy and weakness, and consequently reduced maximal muscle strength has been observed in patients with T2DM compared to age-matched healthy controls. This could be partially explained by several T2DM-induced alterations in the neuromuscular system, such as changes in the α-motor neurons, neuromuscular junction and skeletal muscle fibers. These pathological changes in properties and number are correlated with age and the T2DM disease duration [27-29]. When dNP is finally detected, this complication can be associated with even greater changes in the neuromuscular system and motor dysfunction, which directly leads to increased muscle atrophy and weakness, the slowing of muscle contractile properties, reduced motor unit firing rates, and motor unit loss [66].

Comparison between patients with T2DM and healthy controls

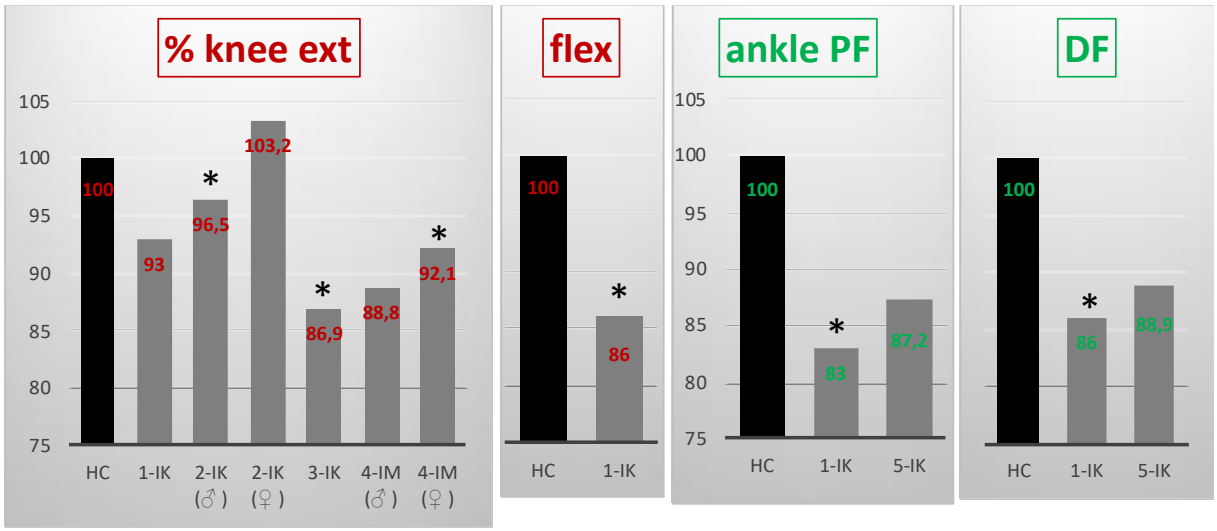
Solely looking at the upper limb assessments, one study reported on significant lower mean IM Handgrip Strength (HGS) of -13.4% in the total T2DM group versus the healthy controls (HC) [79].

Exploring research on the lower limb only, one study was found which revealed significant lower IM maximal muscle strength data on the knee extensors and ankle plantar flexors in patients with T2DM versus HC (respectively -31.6% and -34%) [67].

Furthermore, five articles reported on IM or IK maximal muscle strength of both upper (HGS, wrist and/or elbow) and lower (ankle and/or knee) limb (see Figure 3). However, no comparisons were made between maximal muscle strength results of the upper

versus the lower limbs. These five articles generally revealed a 10-20% decline in maximal muscle strength in the tested joints of patients with T2DM compared to healthy controls. Nevertheless, not all results showed significant differences [55-59].

ASSESSMENTS OF **KNEE (ext-flex)** AND **ANKLE (PF-DF)** [55-59]



ASSESSMENTS OF **HANDGRIP**, **ELBOW (ext-flex)** AND **WRIST (PF-DF)** [55-59]

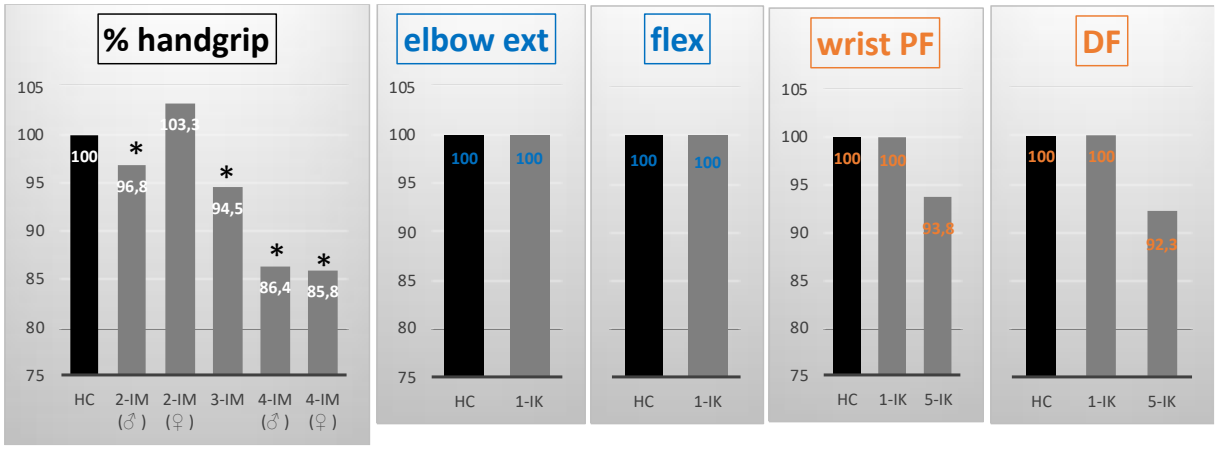


Figure 3 HC (■), healthy controls = 100%; ■, patients with T2DM; ext, extension; flex, flexion; PF, plantar/palmar flexion; DF, dorsiflexion; IK, isokinetic; IM, isometric.
1, Andersen et al., 2004 [55]; 2, Park et al. 2006 [56]; 3, Park et al. 2007 [57]; 4, Guerrero et al., 2016 [58]; 5, Andreassen et al., 2006 [59].

* $p<0.05$

Comparison between T2DM patients without dNP and healthy controls

Two studies reported on significant lower IM maximal knee extension and flexion strength between dNP- and HC [49, 72]. In one of these studies the same outcome was observed when measuring IK maximal muscle strength [49]. Concerning IM ankle plantar and dorsiflexion, the data of two studies were consistent with significant lower maximal muscle strength in dNP- versus HC, except for ankle dorsiflexion in one study [49, 71]. IK maximal muscle strength in ankle plantar and dorsiflexion showed overall significant lower values in dNP- versus HC [49, 68].

Comparison between T2DM patients with dNP and healthy controls

Two of the former mentioned studies, reporting on IM maximal knee extension and flexion strength, showed also significant lower values between dNP+ and HC [49, 72]. Moreover, IK maximal muscle strength measurements revealed the same outcome in knee extension as well as in flexion [49]. Concerning IM maximal ankle muscle strength, three out of seven researchers investigated maximal plantar flexion strength, which revealed significant lower values in the dNP+ group compared to HC [64, 69, 93]. Similarly, in all seven studies, IM maximal dorsiflexion strength was measured, again revealing overall significant lower values in the dNP+ group compared to HC [29, 49, 64, 65, 69, 93, 94]. Concerning IK maximal ankle muscle strength, two researchers reported significant lower results in dNP+ versus HC for all movement directions [49, 68].

Comparison between T2DM patients with dNP and without dNP

Concerning upper limb investigation only, one IM study mentioned no significant differences between dNP+ and dNP- [79]. One investigator performed IK maximal muscle strength tests on wrist palmar and dorsiflexion and ankle plantar and dorsiflexion without significant differences between the two diabetes subgroups (see also Figure 3; 5-IK) [59].

For IM maximal muscle strength of the knee extensors, significant lower values were found in dNP+ versus dNP- (-33.3%) [67]. Results on both IM and IK knee extension/flexion and ankle plantar/dorsiflexion exposed no significant differences between dNP+ and dNP- [49].

Heterogeneity of the studies on peripheral maximal muscle strength

It is worthwhile to mention that all studies regarding maximal muscle strength in the upper and lower limbs of T2DM patients without or with dNP, reveal a high level of

heterogeneity in assessing, reviewing, and analyzing the results. In most of the cases, this is due to the discrepancy in the study methods used to detect dNP, such as unconfirmed dNP, clinically diagnosed dNP, and dNP based on ENMG, but also in different strength measurements (IM versus IK; non-dominant versus dominant upper or lower limb), and in selected equipment and performed tests (physical function/fitness laboratory testing (such as dynamometry and mechanography) versus physical performance field testing (such as Short Physical Performance Battery test, Chair Rising Test and Timed Up and Go)) [82, 83]. Last but not least, statistical data analyses were carried out on absolute values in one study and on relative values in the other. However, as there is a direct relation between lean body mass and muscle strength, larger individuals generally have more lean body mass, and therefore have the capacity to generate greater strength. For this reason, muscle strength should preferably be expressed in relative terms [84].

3.2.2. Muscle endurance

In contrast to the well-known consequences of the alterations in the neuromuscular system of patients with dNP concerning maximal muscle strength, little is known regarding how these alterations may affect the performance of the neuromuscular system when its capacity is stressed during a fatiguing task and subsequent recovery [66]. Healthy ageing has been associated with greater resistance to fatigue in certain isometric tasks and muscle groups, but not in all of them. It is not entirely clear whether slowed contractile properties and metabolic functions, often reported in the healthy ageing population and in patients with dNP, necessarily provide greater muscle fatigue [29, 95]. Therefore, it is an interesting model to study the potentially different impact of dNPs and dNPsm on muscle endurance and fatigue.

Comparison between T2DM patients without (and with) dNP, and healthy controls

To our knowledge, only two research groups investigated muscle endurance, both only in the lower limbs. Allen et al., 2015, reported a 21% decline in the ankle DF endurance test of patients with dNP+ compared to healthy controls [66]. IJzerman et al., 2012, investigated the muscle endurance of knee extensors/flexors and ankle PF/DF in patients with dNP-, with dNP+, and healthy controls. The only significant lower value was observed for fatigue knee flexion in the dNP- group versus the healthy controls [72], implying ambiguous data.

3.2.3. Explosive muscle strength

The influence of T2DM and dNP on explosive muscle strength remains unexplored. Previous research in older adults highlighted the importance of assessing muscle power/function and the integration with the neural coordination of the muscle contraction in complex physiological movements such as jumping or sit-to-stand against gravity (e.g. by means of neuromuscular performance tests applying mechanography), as this has been shown a robust indicator and a relevant correlate for functional decline, postural instability and age-associated fall risk [96]. Yet, it could be of interest to gain information on explosive muscle strength in T2DM patients without dNP, and with sensory and sensorimotor dNP in order to identify those who are at high risk for functional decline and in order to monitor and evaluate preventive strategies for combating decreased functional performance, ADL, (in)dependence and increased risk of falls [96, 97].

4. Respiratory muscle strength

4.1. Definitions and assessments

Patients with T2DM may present pulmonary function abnormalities caused by long-term exposure to hyperglycemia [53]. These abnormalities may include respiratory muscle impairment [53], which is commonly associated with dNP [52-54, 98, 99], as well as reduction in lung volumes, carbon monoxide diffusion, decreased pulmonary compliance, and lung elastic recoil [52, 53]. So, as respiratory muscle weakness in patients with T2DM may often been associated with peripheral dNP [98] and/or autonomic cardiovascular dNP [52, 54, 99], the assessment of respiratory muscle strength in the population groups without and with peripheral dNP (dNP- and dNP+) is another important point of interest within our research domain.

In order to fully understand why researchers describe two different types of pulmonary dNP, the innervation of the respiratory muscles in charge of in- and expiration, has to be explored in depth. While breathing in, the inspiratory muscles (including the diaphragm, the sternocleidomastoid, the scalene muscles, and the external intercostal muscles) contract by recruiting non-volitional spinal nerves C3, C4 and C5 (the phrenic nerve) which innervate the diaphragm, the cranial nerve XI, spinal nerves C1 and C2 which innervate the sternocleidomastoid and the scalene muscles, and the spinal nerves T1 to T12 which innervate the external intercostal muscles. Inspiration is also facilitated by skeletal auxiliary respiratory muscles (such as the pectoralis major and

minor, serratus anterior, and latissimus dorsi) driven by large myelinated Aα fibers. Contrary to the inspiration progress, expiration by means of quietly breathing out is rather a passive process by relaxing the diaphragm. However, when performing expulsive efforts, such as coughing, vomiting, sneezing and defecation, the internal intercostal and abdominal muscles are being mobilized. The internal intercostals are innervated by the spinal nerves T1 to T12 and the abdominals by spinal nerves T7 to L1. Generally, all respiratory muscles are controlled by the respiratory centers of the autonomic nervous system in the pons and medulla oblongata, and are depending on intact motor nerve supply, comparable to all skeletal muscles [100, 101].

Therefore, in this dissertation, the decline in respiratory muscle strength was also investigated as a combination of autonomic and somatic dNP can be present.

Most research has been conducted by assessing respiratory muscle strength (maximal static inspiratory and expiratory pressure measurements (PI_{max} and PE_{max}; cm H_2O)) in combination with pulmonary function (peak expiratory flow (PEF; L/m)), as both parameters are strongly associated. PI_{max} and PE_{max}, produced against an occluded airway, are the most widely used methods to gauge respiratory muscle strength and to determine respiratory muscle weakness as a result of ageing or caused by certain chronic diseases, such as T2DM [102]. Furthermore, PEF is an internationally recognized tool to evaluate pulmonary function in subjects without lung diseases [103].

4.2. State of the art in patients with diabetic neuropathy

The association between reduced pulmonary function and T2DM has been scarcely described in previous literature, without revealing apparent underlying mechanisms and significant dependent variables (e.g. severity and duration of T2DM, obesity, smoking habits, levels of HbA1c, …) [104-106]. Three studies claimed that respiratory muscle weakness in patients with T2DM may occur and might be associated with autonomic dysfunction [52, 54, 99]. Only one study, conducted by using invasive non-volitional phrenic nerve stimulation, revealed impaired respiratory neuromuscular function in patients with T2DM, which is strongly related to peripheral dNP [98]. Due to the limited amount of research on the impact of peripheral dNP on respiratory muscle strength and pulmonary function, we decided to initiate more in-depth research on D- versus D+ patients.

5. Summary

As skeletal muscle strength is an important parameter of physical function/fitness, it may have large impact on the level of physical activity and functioning. Ageing and several chronic disorders (T2DM amongst others) also have a strong negative impact on respiratory muscle strength and will limit physical capacity. Some diabetic subgroups suffer from multiple risk factors that might impact physical capacity even more. However, data in literature is very scarce and skeletal muscle strength research has only been performed on the total T2DM patient group. Only in few studies, maximal muscle strength has been investigated in different dNP subgroups. Further elaborated information on peripheral dNP does not exist. However, this exploration is mandatory in the prevention of muscle strength decline and functional dependency. Moreover, with this extra knowledge, physical training programs could be adapted and adjusted to the patients' progression and needs to focus on the restoration of their functionality and to maintain their common-dwelling nature.

6. Aims and main research questions

Based on the rationale mentioned above, the aim of our original research is to gain more insight in the impact of diabetic neuropathy (sensory and sensorimotor dNP), on muscle strength (respiratory and peripheral) in patients with T2DM. As skeletal muscle weakness in this population affects physical functioning, training programs should be implemented in order to maintain or improve appropriate muscle strength and, consequently, physical function/fitness and functional performance. Hence, more research is mandatory to optimize such training programs.

First aim: The influence of clinically diagnosed neuropathy on respiratory muscle strength in type 2 diabetes mellitus.

This first part of the original research was conducted on patients with T2DM, without and with dNP, defined by clinical neurological examination only (without ENMG confirmation) compared to healthy controls.

To answer the **first** research aim, respiratory muscle strength and function were assessed by measuring maximal inspiratory and expiratory mouth pressures, and respiratory function by peak expiratory flow. We hypothesized that, compared to healthy controls, respiratory muscle strength and function could be decreased in T2DM patients without dNP and even more impaired by the presence of dNP.

Second aim: The impact of sensory and/or sensorimotor neuropathy on lower limb muscle endurance, explosive and maximal muscle strength in patients with type 2 diabetes mellitus.

Third aim: The impact of diabetic neuropathy on the distal versus proximal comparison of weakness in lower and upper limb muscles of patients with type 2 diabetes mellitus: a cross-sectional study.

This second and third part of the original research were conducted on T2DM patients without dNP, with sensory dNP, with sensorimotor dNP, and healthy controls without neuropathy, with a classification based on ENMG.

The **second** part consisted of the impact of sensory and/or sensorimotor neuropathy on lower limb muscle endurance, explosive and maximal muscle strength in patients with T2DM. Hence, we hypothesized that both muscle endurance and explosive muscle strength should be affected in T2DM patients without and with neuropathy compared to a healthy control group in the same age category, and that this affection should increment from patients without diabetic neuropathy, over patients with sensory diabetic neuropathy into patients with sensorimotor diabetic neuropathy.

The **third** part of the research was conducted in the same population group, in order to determine whether muscle weakness was more pronounced in the distal (ankle) compared to proximal (knee) part of the lower limb and in the distal (hands) compared to proximal (elbow) part of the upper limb in patients with T2DM and, if present, whether this was affected by the presence and severity of dNP.

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PART II

ORIGINAL RESEARCH

1.

The Influence of Clinically Diagnosed Neuropathy on Respiratory Muscle Strength in Type 2 Diabetes Mellitus

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Abstract

Objectives: This cross-sectional study investigated the influence of clinically diagnosed neuropathy (cdNP) on respiratory muscle strength in patients with type 2 diabetes mellitus (T2DM).

Methods: 110 T2DM patients and 35 non-diabetic healthy controls (≥60 years) were allocated to one of three groups depending on the presence of cdNP: T2DM without cdNP (D-; n=28), T2DM with cdNP (D+; n=82) and controls without cdNP (C; n=35). Clinical neurological diagnostic examination consisted of Vibration Perception Threshold and Diabetic Neuropathy Symptom score. Respiratory muscle strength was registered by maximal Inspiratory and Expiratory Pressures (PI_{max} and PE_{max}), and respiratory function by Peak Expiratory Flow (PEF). Isometric Handgrip Strength and Short Physical Performance Battery were used to evaluate peripheral skeletal muscle strength and physical performance. Univariate analysis of covariance was used with age, level of physical activity and Body Mass Index as covariates.

Results: PI_{max}, PE_{max} and PEF were higher in C compared to D- and D+. Exploring more in detail, PI_{max}, PE_{max} and PEF were significantly lower in D+ compared to C. PE_{max} and PEF were also significantly lower in D- versus C. Measures of peripheral muscle strength and physical performance showed less associations with cdNP and T2DM.

Conclusions: The presence of cdNP affects respiratory muscle strength in T2DM patients compared to healthy controls. Both cdNP and diabetes in themselves, showed a distinctive impact on respiratory muscle strength and function, however, an accumulating effect could not be ascertained in this study. As commonly used measures of peripheral muscle strength and physical performance seemed to be less affected at the given time, the integration of PI_{max}, PE_{max} and PEF measurements in the assessment of respiratory muscle weakness could be of added value in the (early) screening for neuropathy in patients with T2DM.

Key Words: Diabetes Mellitus, Neuropathy, Respiratory Muscle Strength, PI_{max}, PE_{max}, PEF

Introduction

Type 2 Diabetes Mellitus (T2DM) is the most common cause of (sensori)motor and autonomic neuropathy [1, 2]. One of the most important and well-recognized clinical manifestations of diabetes-associated neuropathy (NP) is impairment and debilitation in functioning and locomotion due to the development of lower limb skeletal muscle weakness, which is closely related to the severity of NP [3, 4]. Studies using Magnetic Resonance Imaging (MRI) showed accelerated muscle atrophy in accordance with increased loss of muscle strength in patients with T2DM suffering from symptomatic NP in comparison to T2DM patients without NP and healthy controls [5, 6].

For that matter and from a clinical point of view, the assessment of the influence of NP on muscle function is highly recommended and mainly achieved by means of standardized clinical examinations such as manual muscle testing, isometric and isokinetic dynamometry, Handgrip Strength (HGS) and by functional performance tools (e.g. timed Chair Stand Test (CST) and indirectly by gait analysis and appraisal) [5, 7-9].

Approximately 10-15% of all people aged >40 suffers from NP in which diabetes remains the most common cause. Asides age, diabetes and a set of other distinctive factors causing NP has to be classified as idiopathic in 20-30% of all patients suffering from NP, even after thorough investigation. This idiopathic NP is considered as a major culprit of a person's disability with important social impact due to pain, gait instability, increased risk of falls, injuries and poor quality of life [10-12].

The association between reduced respiratory function and T2DM has already been described [13], however, the underlying mechanisms are still undisclosed. Klein et al. reported in a systematic review an inverse association between respiratory function on the one hand and blood glucose levels, the severity and duration of T2DM on the other, independent of smoking status or presence of obesity [14]. Van den Borst et al. also reported a decreased lung function in T2DM. Meta-regression analysis showed, however, that this relation could not be explained by Body Mass Index (BMI), smoking, diabetes duration, or glycated hemoglobin (HbA1c) [15]. Respiratory muscle strength is strongly associated with pulmonary function and may play important roles in the respiratory network, which on its turn depends on intact neural circuitry that orchestrates the interplay between respiratory muscles and intrinsic pulmonary function to maintain adequate ventilation [16]. Kabitz et al. showed that impaired

respiratory neuromuscular function, which is strongly related to diabetic polyneuropathy, occurs in T2DM patients as assessed by non-volitional gold-standard phrenic nerve stimulation [4]. Also other studies have reported that in patients with T2DM, respiratory muscle weakness can occur and might be associated with autonomic dysfunction [17, 18]. In contrast to the large number of studies examining peripheral muscle weakness in T2DM patients with NP [3, 5, 8, 19-22], only limited research has been conducted regarding the impact of diabetic –or any kind for that matter– NP on respiratory muscle strength [4].

The aim of the present study is to evaluate respiratory muscle strength and function in T2DM patients with clinically diagnosed neuropathy (cdNP) and to compare this with T2DM patients without cdNP and healthy controls. We hypothesize that, compared to healthy controls, respiratory muscle strength and function are decreased in T2DM patients without cdNP and even more impaired in the presence of cdNP.

With respect to the aforementioned hypothesis, the assessment of maximal static Inspiratory and Expiratory Pressure measurements, and Peak Expiratory Flow could be considered regarding its added value in the screening for NP.

Materials and Methods

Study Design and Population

In this cross-sectional case-control study 110 patients with T2DM and 35 healthy controls were included (n=145).

Participants comprised both community-dwelling elderly and elderly living in a residential care setting. Patients with T2DM were recruited through the Department of Endocrinology (University Hospital Ghent) and their general practitioner, and healthy controls by online advertising and flyer distribution. T2DM was diagnosed on two different occasions based on HbA1c assessments according to the Type 2 Diabetes ADA Diagnosis Criteria [23].

Criteria for inclusion were: (i) aged 60 years or more, (ii) living in the community or residential care setting, (iii) able to respond adequately to Dutch instructions and (iv) able to walk independently with or without walking aids. Subjects suffering from (i) major neurological conditions (e.g. stroke, Parkinson's disease, dementia), (ii) musculoskeletal disabilities (e.g. foot ulcerations, lower limb amputations, arthritis with

limited joint mobility precluding ambulation), (iii) severe cardiovascular disorders (e.g. exercise-induced chest pain, Congestive Heart Failure (New York Heart Association class III and IV)) and (iv) respiratory diseases (e.g. exercise-induced asthma, COPD (Global Initiative for Chronic Obstructive Lung Diseases (GOLD)) stages 3 and 4) were excluded.

Based on a clinical neurological diagnostic examination performed by trained physical therapists, the population could be divided into three groups: T2DM without cdNP (D-; n=28), T2DM with cdNP (D+; n=82) and non-diabetic healthy controls without cdNP (C; n=35). Control subjects having NP after the clinical neurological examination were excluded as the differentiating etiology of NP was not further examined.

The Ethical Committee of the Ghent University Hospital gave approval to this study and all participants signed an informed consent. Consequently, this research is compliant with the World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects.

Outcome Measurements

All measurements were performed on a single morning in a quiet setting and well-lit room with flat surface. At first, anthropometrical data and HbA1c were obtained followed by NP-examinations. Subsequently, respiratory muscle strength, HGS and the Short Physical Performance Battery (SPPB) were assessed and a physical activity questionnaire was completed.

Patient Characteristics

Height, weight, BMI and body composition (bioelectrical impedance analysis; BIA, Bodystat® 1500MDD) were measured and calculated.

HbA1c was measured using the A1CNow SELFCHECK® (Bayer), an instrument which is well correlated to standardized laboratory HbA1c tests (r=0.758) [24].

Habitual physical activity levels were measured using the physical activity questionnaire for the elderly [25, 26].

Measurements of Peripheral Clinically Diagnosed Neuropathy

The clinical neurological diagnostic examination consisted of two parts: measurements of the Vibration Perception Threshold (VPT), an assessment of the peripheral large-

fiber sensory nerve function, and the Diabetic Neuropathy Symptom score questionnaire (DNS).

The VPT, a valid and reliable measurement was determined using a Bio-Thesiometer® (Bio Medical Instrument co, Ohio, USA) on the left and right medial malleolus and on the distal plantar surface of the big toes [27, 28]. VPT was defined as the lowest recorded voltage when subjects indicated the sense of vibration. Each measurement was repeated three times and the lowest reading was considered [29, 30]. Since the threshold at which vibration becomes perceptible is dependent of age, gender and location, four percentile rank charts of VPT variation were used [29]. To decide whether vibration perception was within the normal range, a normality cut-off on the 95th percentile was applied. If one of the readings (big toe and the medial malleolus, both left and right) was above the 95th percentile, this criterion was classified as positive.

The DNS, a validated 4-point yes/no questionnaire, has a high predictive value in the screening for diabetic NP when patients score ≥1/4. Meijer et al. compared the validity, predictive value and reproducibility of the DNS with the Neuropathy Symptom Score (NSS). They found a high correlation between NSS and DNS score (r=0.88) and concluded that the DNS is a fast, easy, reproducible (κ 0.78-0.95) and valid assessment tool to screen for diabetic polyneuropathy [31].

Patients with T2DM were classified as having peripheral cdNP based on at least one of two positive criteria: a VPT exceeding the normality cut-off of 95% or a DNS score of ≥1/4.

Measurements of Muscle Strength

The maximal static Inspiratory and Expiratory Pressure measurements (PI_{max} and PE_{max}; cm H2O) were obtained by a Pocket-Spiro Mouth Pressure Monitor with a differential pressure transducer (MPM100; Medical Electronic Construction®). To measure PI_{max}, subjects were seated and asked to exhale slowly and completely up to residual volume and then to perform a maximum inspiratory maneuver during at least 1.5 seconds against a completely occluded airway. Then, a 1-second average including the peak pressure was calculated, indicating the inspiratory muscle strength. PE_{max} was determined under the same conditions while first inhaling completely up to total lung capacity and then performing a maximum expiratory maneuver. For each index, three tests were recorded and the highest value was used for data analysis [32-

[35]. The measurement of the maximum static mouth pressures produced against an occluded airway, is the most widely used method of measurement and is an easy way to gauge respiratory muscle strength and to determine the severity of respiratory muscle strength impairments [36]. Additionally, Peak Expiratory Flow (PEF; L/m), a cheap, simple and widely accessible technique with a prognostic value for morbidity [37, 38], was recorded using a Mini-Wright Peak Flow Meter (Henrotech®). This is internationally recognized as the golden standard for PEF measurements [39]. PEF is used as indicator for respiratory muscles strength in subjects without lung disorders [37].

Isometric HGS (kg) was measured according to the American Society of Hand Therapists guidelines [40] using the Jamar® dynamometer (Sammsons Preston Rolyan Inc., Bolingbrook, IL) at the dominant side [37]. The highest grip score of three consecutive trials was retained.

Measurements of Physical Performance

The SPPB consists of a timed standing balance test (feet together side-by-side, semi-tandem and tandem stance), a walk test (time to walk 2.44 meters at usual pace) and a CST (time to raise from a chair and return to the seated position in five times) [41]. Each of the three component tasks was rated from 0 (unable to complete) to 4 (best), and a compiled score was computed by the sum of scores on component tasks (range 0=worst to 12=best) [42, 43]. This composite test is often used and validated as a standard assessment of physical performance in research and clinical practice of the ageing population [37, 44].

Statistical Analysis

Data were analyzed using IBM Statistical Package for Social Sciences (SPSS version 24 for Windows) and were considered significant at $\alpha<0.05$. After confirming the approximate normality of data using the Shapiro–Wilk test, descriptive statistics for anthropometric, biochemical and respiratory muscle parameters are presented by arithmetic mean (standard deviation; SD), median (min-max) and by ratio (% and count). Between-groups analysis was performed using univariate analysis of covariance (ANCOVA) with age, level of physical activity and BMI as covariates. Post hoc comparisons were corrected with the Bonferroni test. A Pearson Chi-Square test was used for gender and residential status in order to detect all between-group differences.

Linear regression analysis between VPT, DNS, HbA1c and age on the one hand and measures of respiratory muscle strength and function (i.e. PI_{max}, PE_{max} and PEF) on the other was performed.

Results

Subject characteristics are shown in Table 1. The healthy control group was significantly younger (F=3.487; *p*=0.017), had lower BMI (F=3.561; *p*=0.015), was more physically active (F=5.343; *p*=0.002) and proportionally a minority was living in a residential setting (*p*=0.002) compared to the other groups. There was no significant difference in gender distribution between the 3 groups (*p*=0.587).

HbA1c levels were significantly higher in the diabetes groups compared to the control group (F=24.894; *p*<0.001), but showed no significant differences between the diabetes groups (D- vs D+). Also no significant between-group differences were found for duration of diabetes.

Table 1: Subject characteristics

	C (n=35)	**D-** (n=28)	**D+** (n=82)
Age *(yrs)*	73 (6.8)	79 (9.9)	**79 (9.1)[a]**
BMI *(kg/m²)*	28 (4.0)	**31 (6.2)[a]**	29 (5.3)
HbA1c *(%)*	5.5 (0,40)	**6.7 (0.81)[a]**	**6.7 (1.24)[a]**
Diabetes duration *(yrs)*	/	10.5 (8.34)	10.3 (8.64)
Level of Physical Activity	8.2 (1.24-32.19)	6.4 (0.00-38,35)	**2.6 (0.00-36.41)[ab]**
Male : Female			
(%)	43 : 57	39 : 61	34 : 66
(count)	15 : 20	11 : 17	28 : 54
Community-dwelling : RCS			
(%)	71 : 29	**29 : 71[a]**	**37 : 63[a]**
(count)	25 : 10	**8 : 20[a]**	**30 : 52[a]**

Data were expressed as mean (SD), with exception for level of physical activity as median (min-max), gender and residential status as ratio (% and count).

C, healthy controls; D-, T2DM without cdNP; D+, T2DM with cdNP; yrs, years; RCS, Residential Care Setting; HbA1c, glycated hemoglobin.

a *p*<0.05 compared to C

b *p*<0.05 compared to D-

VPT-toe measures (left versus right) and VPT-ankle measures in the C, D- and D+ are presented in Table 2.

Table 2: VPT scores

VPT: highest Voltage of the left versus right toe			
	C (n=34**)**	**D-** (n=26)	**D+** (n=74)
means (SD)	20.2 (5.54)	20.3 (6.04)	37.5 (12.26)
min-max	10-38	10-34	10-50
VPT: highest Voltage of the left versus right ankle			
	C (n=34**)**	**D-** (n=26)	**D+** (n=70)
means (SD)	23.6 (6.50)	24.0 (7.36)	40.6 (11.60)
min-max	12-35	10-45	7-50

Data were expressed as mean (SD) and minimum-maximum (min-max); VPT, Vibration Perception Threshold.

C, healthy controls; D-, T2DM without cdNP; D+, T2DM with cdNP.

The actual values of DNS reveal a score of zero on the scale of 0-4 in C in contrast with D+ (Table 3).

Table 3: DNS scores

	C (n=26)	**D-** (n=22)	**D+** (n=70)
means (SD)	0 (0)	0 (0)	1.4 (1.35)
median (min-max)	0 (0-0)	0 (0-0)	1 (0-4)

Data were expressed as mean (SD) and median (minimum-maximum).

DNS, Diabetic Neuropathy Symptom score; C, healthy controls; D-, T2DM without cdNP; D+, T2DM with cdNP.

Linear regression analysis between VPT, DNS, HbA1c and age on the one hand and measures of respiratory muscle strength and function (i.e. PI_{max}, PE_{max} and PEF) on the other has been performed. Linear regression analysis on the respiratory muscle strength (i.e. PI_{max} and PE_{max}) shows that the VPT scores are the only significant explanatory values for the variances, respectively in PI_{max} (8.2%) and PE_{max} (10.9%). Analyzing respiratory function (i.e. PEF), VPT (2.8%), Hb1Ac (5.5%) and age (17.5%) significantly explain 25.8% of the variance in PEF. The outcome data are documented in Table 4.

Table 4: Linear regression analysis between VPT, DNS, HbA1c and age on one hand, and PI_{max}, PE_{max} and PEF on the other

	PI_{max}	**PE_{max}**	**PEF**
Explanatory variables	- VPT	- VPT	- VPT - HbA1c - Age
Adjusted R square	0.082	0.109	0.258
***p*-values**	*p*=0.003	*p*=0.001	*p*<0.001

PI_{max}, Maximum Inspiratory Pressure; PE_{max}, Maximum Expiratory Pressure; PEF, Peak Expiratory Flow; VPT, Vibration Perception Threshold; HbA1c, glycated hemoglobin.

Table 5 reports on the assessment of respiratory muscle strength between the three groups, corrected for age, physical activity and BMI. Significant differences were observed for PI_{max}, PE_{max} and PEF. Post hoc analyses revealed significant lower values in D+ compared to C for PI_{max} (*p*=0.005), PE_{max} (*p*=0.001) and PEF ((*p*<0.001). When comparing D- with C, only PE_{max} (*p*=0.039) and PEF (*p*=0.026) were significantly lower.

Table 5: Univariate analysis of covariance (ANCOVA, corrected for age, body mass index and physical activity) on respiratory muscle strength and function

	F-value ***p*-value**	**C** (n=35)	**D**- (n=28)	**D+** (n=82)
PI_{max} (cm H_2O)	F=5.289 *p*=0.007	64.5 (28.83)	40.7 (25.22)	**36.6 (23.71)[a]**
PE_{max} (cm H_2O)	F=6.785 *p*=0.002	100.6 (29.58)	**69.5 (29.97)[a]**	**65.2 (31.20)[a]**
PEF (L/min)	F=10.600 *p*=0.001	471.2 (132.27)	**330.9 (152.07)[a]**	**314.5 (221.26)[a]**

Data were expressed as mean (SD).

C, healthy controls; D-, T2DM without cdNP; D+, T2DM with cdNP; PI_{max}, Maximum Inspiratory Pressure; PE_{max}, Maximum Expiratory Pressure; PEF, Peak Expiratory Flow.

a *p*<0.05 compared to C

Functional assessment data (i.e. HGS and SPPB) between the three groups are presented in Table 6, corrected for age, physical activity and BMI.

HGS revealed no between-groups differences (F=2.100; *p*=0.128). Statistical significant differences were observed on both the SPPB total (F=7.209; *p*=0.001) as in its subdomains; CST (F=4.533; *p*=0.013), balance (F=3.835; *p*=0.025) and gait (F=4.130; *p*=0.019) with better performance in favor of C. For SPPB total and SPPB gait, post hoc analysis revealed significant higher values in C compared to D- (*p*=0.008 and *p*=0.043 respectively) and D+ (*p*=0.002 and *p*=0.031 respectively). CST and balance subdomains showed significant better scores for C compared to D+ (*p*=0.010 and *p*=0.028 respectively). Considering SPPB balance, only tandem stance showed significant higher results comparing C to D+ (*p*=0.019).

Table 6: Univariate analysis of covariance (ANCOVA, corrected for age, body mass index and physical activity) on peripheral muscle strength, balance and gait

	F-value *p*-**value**	**C** (n=35)	**D-** (n=28)	**D+** (n=82)
HGS (kg)	F=2.100 *p*=0.128	26.9 (12.36)	20.1 (10.15)	17.6 (9.50)
SPPB: total	F=7.209 *p*=0.001	11 (4-12)	**7 (1-12)[a]**	**6 (1-12)[a]**
A. CST	F=4.533 *p*=0.013	3 (0-4)	1 (0-4)	**0 (0-4)[a]**
B. balance total	F=3.835 *p*=0.025	4 (3-4)	3,5 (0-4)	**3 (0-4)[a]**
side-by-side	F=0.508 *p*=0.603	2 (2-2)	2 (0-2)	2 (0-2)
semi-tandem	F=1.334 *p*=0.268	2 (2-2)	2 (0-2)	2 (0-2)
tandem	F=3.966 *p*=0.022	2 (1-2)	1,5 (0-2)	**1 (0-2)[a]**
C. gait	F=4.130 *p*=0.019	4 (1-4)	**2 (1-4)[a]**	**3 (1-4)[a]**

Data were expressed as median (min-max), with exception for HGS as mean (SD).

C, healthy controls; D-, T2DM without cdNP; D+, T2DM with cdNP; HGS, Handgrip Strength; SPPB, Short Physical Performance Battery; CST, Chair Stand Test.

a *p*<0.05 compared to C

Discussion

The present study was conducted to investigate respiratory muscle strength and function in T2DM and its relation to NP by comparing PI_{max}, PE_{max} and PEF between T2DM patients with cdNP, T2DM patients without cdNP and healthy controls.

The key findings of this study were lower measures of PI_{max}, PE_{max} and PEF in the D- and D+ groups compared to C.

Looking more in detail to the results, all three respiratory muscle outcomes were significantly lower when comparing D+ to C, PE_{max} and PEF were significantly lower in D- and D+ compared to C. Herewith, it seems that the presence of NP as well as T2DM has an impact on respiratory muscle outcome. However, an accumulating effect of cdNP and T2DM could not be ascertained.

A posteriori power calculation on respiratory muscle strength and function (PI_{max}, PE_{max} and PEF) resulted in a power of 0.826, 0.912 and 0.927 respectively.

To understand our NP-related results, the innervation of the respiratory muscles should be explored in depth. While breathing in, the inspiratory muscles contract by recruiting non-volitional spinal nerves C3, C4 and C5 (the phrenic nerve) innervating the diaphragm, cranial nerve XI, spinal nerves C1 and C2 innervating the sternocleidomastoid and the scalene muscles, and T1 to T12 for the external intercostal muscles. The two expiratory muscle groups (the internal intercostals and abdominals) are usually not used during quiet breathing, but are essential in performing expulsive efforts, including cough, vomiting and defecation. Due to their character, these expiratory muscles are of utmost importance during forced expiration (such as in PE_{max} and PEF, during static and dynamic trunk control, and Valsalva maneuvers). The internal intercostal muscles are innervated by the spinal nerves T1 to T12 and the abdominals by spinal nerves T7 to L1.

The respiratory muscles are generally controlled by the respiratory centers of the autonomic nervous system in the pons and medulla oblongata, and are depending on intact motor nerve supply, comparable to all skeletal muscles [45, 46].

Kabitz et al. used bilateral anterior magnetic phrenic nerve stimulation whereby the cortical motor command was bypassed in order to assess respiratory neuromuscular function related to diabetic polyneuropathy in patients with T2DM [4]. They provided the

first data regarding cdNP and concluded that cdNP was associated with substantially impaired respiratory neuromuscular function in patients with T2DM, when stimulating the non-volitional phrenic nerve. No alterations in respiratory function could be found when assessing volitional respiratory muscle strength. The volitional respiratory neuromuscular function testing was performed by using PI_{max}, PE_{max} and maximal sniff pressures. PEF, however, was not assessed in this particular study. The exclusion criterion used by Kabitz et al. consisted in the diabetic group of known primary lung diseases, whereas in the control group healthy male subjects experiencing lung, cardiac or metabolic diseases were excluded [4]. The different eligibility criteria between the research of Kabitz et al. and our own study could explain the different results. We opted for stricter selection criteria such as exclusion of patients with exercise-induced asthma, and COPD GOLD stages 3 and 4. It is also of importance to mention the lower mean age of their controls (60.3 years ± 6.9) and the diabetic patients (63.6 years ± 7.5) compared to the present study, which could explain the differences in outcome of the volitional tests on respiratory neuromuscular function [47].

To understand the impact of T2DM as such, we have to focus on muscle mass, muscle fiber type distribution and vascularization. Checking on the muscle fiber type distribution of the diaphragm in healthy humans, the mean relative occurrence of Slow Twitch fibers (type I) is approximately 50%. The remaining proportion is equally divided into two different Fast Twitch fibers (type IIa and IIx). Both the inspiratory and expiratory intercostal muscles have at least 10% more type I fibers than the diaphragm and most other skeletal muscles, whereas the expiratory internal intercostal muscles show an almost complete absence of type IIx fibers [46].

In healthy humans, all muscle fibers are surrounded by a certain number of capillaries, depending on their fiber type. In the diaphragm, type I fibers are surrounded by 4-6 capillaries per fiber, whereas slightly less (3-5) are found around type IIa and IIx fibers. However, the calculated values for the fiber area surrounded by each capillary are smaller in the diaphragm than in lower or upper limb muscles. In the expiratory intercostal muscles, more capillaries are found around both type I and type IIa fibers (5-6) compared to the inspiratory intercostal muscles (4-5) [46].

In the elderly in general and/or older T2DM patients, abnormalities in muscle morphology have been observed [48, 49]. Studies examining ageing and “accelerated” ageing in the older T2DM patients showed reduced muscle mass and a decrease in

muscle fiber size and number compared to younger controls [50-52]. Fiber size differences, particularly in the type II muscle fibers, seemed to be evident between healthy young men, healthy older men and older age-matched T2DM patients, suggesting that type II fibers are more prone to muscle atrophy in the latter groups. When examining muscle capillary density (as a parameter of microvascular function), capillaries tended to be less prevalent in the elderly and/or older T2DM patients, implicating lower muscle capillary density. Dilation of these small capillaries could explain the observed shift in the distribution of vessel size with a relative loss of small vessels [52].

Measures of peripheral muscle strength (HGS) and functional performance (SPPB total with CST, balance and gait as assessment tools) showed a similar profile as the respiratory muscle strength assessments; i.e. C scored better compared to D- and D+.

Analysis of the peripheral muscle strength showed no significant difference in HGS.

SPPB total, showed a significant difference (p=0.001), mainly allocated to gait (usual gait speed over 2m44; p=0.019) since both post hoc analyses showed significant differences of C versus D+ and D-. Walking is a complex motor skill, involving interactions between sensory and motor attributes, but is essentially supported by appropriate muscle strength and balance. Our findings regarding both strength impairment (CST, p=0.013) and total balance changes (p=0.025) with its subtest 'tandem stance' (p=0.022) endorse the earlier research results in T2DM and cdNP on gait [53]. The argument that gait performance could be influenced by T2DM alone (without NP) has to be taken into consideration based on previous findings [2, 9]. Van Sloten et al. suggested that walking in subjects with T2DM was strongly associated with peripheral NP and decreased muscle strength. This associative result could not be established in our study when T2DM patients were compared to controls [9]. It could be hypothesized that T2DM as such has the same detrimental effects as the presence of cdNP in T2DM on functional capacity (SPPB total and gait).

Based on our data, we can but conclude that both T2DM and NP significantly influence respiratory muscle strength and function. It was, however, not possible to distinct the initial cause (i.e. neuropathic respiratory impairments or diabetes-related pathology) of decreased respiratory muscle strength and function in our T2DM population.

The linear regression analysis on respiratory muscle strength (i.e. PI_{max} and PE_{max}) suggests that the VPT scores are the only significant explanatory values (respectively 8.2% and 10.9% of the variances in PI_{max} and PE_{max}), rather than HbA1c, age and DNS. These results indicate that VPT scores have a larger impact on respiratory muscle strength, supporting the hypothesis that respiratory muscle weakness is due to NP. Analyzing respiratory function (i.e. PEF), VPT (2.8%), Hb1Ac (5.5%) and age (17,5%) significantly explain 25.8% of the variance in PEF. Skloot provided evidence that the ageing process, in the absence of lung disease, alters the intrinsic structure of the lung (changes in collagen fiber network) as well as the supportive extrapulmonary structures (decreased chest wall compliance, reduced curvature of the diaphragm and loss of respiratory muscle mass). These age-related changes in respiratory mechanics lead to a reduction in expiratory flow and lung volumes, and affects lung function [47]. An explanation of the higher impact of HbA1c compared to the VPT scores can be found in the association between increased chronic glycemic exposure to the lung parenchyma and reduced pulmonary function in patients with T2DM [54].

The fact that all existing screening tools and questionnaires only rely on measurements of appendicular muscles, and based on our results, it should be taken into consideration to integrate PI_{max}, PE_{max} and PEF in the screening for respiratory muscle weakness as an indication for the presence of NP in diabetic patients [33]. These findings are supported by Lecube et al. claiming that specific cost-effective screening programs for lung impairment, performed by health care providers, should be investigated in further research [54].

In the clinical practice of a general practitioner, measurements of lung function (forced vital capacity, forced expired volume after 1 second, Tiffeneau-index and PEF) are already regularly applied, mainly in order to detect COPD or other related respiratory disorders. Additional evaluations of respiratory muscle outcomes, which are easy to manage and have low cost impact (e.g. a portable Peak Flow Meter), could be of additional value and of importance in the screening for NP in the T2DM population.

Overall, impairments of respiratory muscle strength and function (PI_{max}, PE_{max} and PEF) were slightly more pronounced compared to those of peripheral muscle strength. Since this study had a cross-sectional design, it was not possible to draw any conclusions concerning the timing of the impact on respiratory or peripheral muscles.

The participants were not questioned concerning smoking condition and alcohol consumption, although this information could have an impact on lung function in general, more specific the PEF values, and on peripheral NP. However, due to a large group of respiratory disorders (astma, COPD GOLD stages 3 and 4) were implemented as exclusion criterion, the impact of lung diseases on PI_{max}, PE_{max} and PEF was significantly reduced. Regarding alcohol consumption, no data were collected which could affect peripheral NP as well. Besides, interviewing subjects about their smoking and drinking habits often leads to ambiguous answers out of exclusion fear.

The cross-sectional design limits drawing conclusions regarding the timing of the impact of causative variables on outcome parameters. Consequently, future research should focus on both longitudinal research and the evolution of physical markers and symptoms such as –in this particular study- onset of diabetes, NP, respiratory and peripheral muscle weakness, etc.

It stands to reason that 82 out of 110 diabetic patients (74,5%) were allocated to the NP group (D+), known as one of the major comorbidities in T2DM patients [1, 2, 55]. Firstly, it is worth mentioning that according to Andersen et al. the prevalence of cdNP increases from 8% in newly diagnosed patients to >40% after 10 years of diabetes [3]. In the present study, the mean duration of diabetes was above 10 years from onset in both D- and D+ groups.

Secondly, the enrolled patients with T2DM showed higher mean age, higher BMI and lower levels of physical activity compared to controls. Ageing is a well-known non-modifiable factor for the development of diabetes and lower muscle strength [50]. BMI has a negative impact on muscle strength in a population with insulin resistance (pre-diabetic situation) and diabetes type 2, which will manifest itself as a decrease of absolute and relative peak torque [56, 57]. Concerning physical activity, low levels have a negative impact on the development of diabetes and on lower muscle strength [57]. In our ANCOVA we encountered this barrier by adding age, BMI and physical activity to add them as covariates.

Finally, peripheral cdNP was diagnosed by VPT in combination with DNS with a deficient comprehensive neurological examination. Hence, we strongly recommend the use of the Michigan Neuropathy Screening Instrument in future research, in order to allocate T2DM patients with/without NP more accurately to the respective groups [58-

[60]. In addition to these clinical assessments, MRI can be used in the detection of symptomatic NP and nerve conduction investigations can be performed by means of electroneuromyography to evaluate sensory action potential amplitude and sensory and motor conduction velocity to confirm the presence and the severity of NP [61]. The main drawbacks to MRI and ENMG techniques are the high costs regarding the number of participants (initially 190) and the subject discomfort.

Conclusions

Based on a substantial population, this research, focusing on respiratory muscle strength, could conclude that this strength is negatively influenced in T2DM patients with and without peripheral neuropathy. A summation effect in patients with diabetes and neuropathy could not be ascertained. Screening for this muscle characteristic may add value to daily clinical practice of T2DM patients in assessment and follow-up.

Data Availability

The data (BirgitVanEetvelde_20180723_revision.zsav) used to support the findings of this study are included within the supplementary information file(s) (available here).

Disclosure

The data of this paper have been presented as a poster at the 54th EASD Annual Meeting, Berlin, Germany, 1-5 October.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Supplementary Materials

Below are template examples that authors may use to write a Data Availability statement. It will often be appropriate to combine templates and edit them as appropriate. The data (BirgitVanEetvelde_20180723_revision.zsav) used to support the findings of this study are included within the supplementary information file(s). Read less. *(Supplementary Materials)*

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2.

The impact of sensory and/or sensorimotor neuropathy on lower limb muscle endurance, explosive and maximal muscle strength in patients with type 2 diabetes mellitus

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Structured Abstract

Aims: The purpose of this study was to investigate the impact of diabetic neuropathy (dNP) on lower limb endurance, explosive and maximal muscle strength in patients with Type 2 Diabetes Mellitus (T2DM).

Methods: Fifty-four participants, aged between 55 and 85, were enrolled in this observational comparative study. The patients with T2DM had an average HbA1c of 7.4% (±1.03) and diabetes duration of 13 years. Participants were classified by means of electroneuromyography as T2DM without dNP (dNP-; n=8), T2DM with sensory dNP (dNPs; n=13), T2DM with sensorimotor dNP (dNPsm; n=14), and healthy controls without neuropathy (C; n=19). Maximal muscle strength and muscle endurance of the dominant knee and ankle were measured by dynamometry, while explosive muscle strength was evaluated by mechanography.

Results: Muscle endurance “total work“ in knee extension and ankle plantar flexion was higher in the healthy controls compared to dNP-, dNPs and dNPsm, in knee flexion compared to dNPs and dNPsm, and in ankle dorsiflexion compared to dNPsm only (p<0.05). Furthermore, relative explosive muscle strength “total power/body weight” and relative maximal muscle strength “peak torque/lean body mass of the dominant leg” considering knee flexion, ankle plantar flexion and dorsiflexion, were higher in healthy controls compared to the dNPsm group, and for maximal muscle strength ankle dorsiflexion even between dNP- and dNPsm (p<0.05).

Conclusions: Muscle endurance is impaired in patients with T2DM, independent of the presence of dNP. Explosive and maximal muscle strength are more likely affected by the presence and severity of dNP.

Key Words: Type 2 Diabetes Mellitus, diabetic neuropathy, maximal muscle strength, muscle endurance, explosive muscle strength

Introduction

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disease, associated with considerable macrovascular (i.e. coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (i.e. diabetic neuropathy (dNP), nephropathy, and retinopathy) with dNP affecting approximately 50% of patients with T2DM [1].

DNP can be classified into sensory dNP (dNPs), characterized by isolated sensory complaints without motor impairment (e.g. reduced tactile function, pain sensation, and proprioception) and sensorimotor dNP (dNPsm), affecting the neuromuscular system and leading to muscle weakness and atrophy of the leg and foot musculature [2].

A considerable body of evidence exists on reduced lower limb maximal muscle strength [3-13] and muscle mass [5-7, 9, 12] in patients with T2DM, with or without dNP, compared to healthy controls. In general, the available studies showed an additive negative effect of dNP, further aggravating the decrease in muscle strength and mass in patients with T2DM. Both T2DM and dNP are associated with metabolic and inflammatory changes that possibly accelerate the age-related deterioration of muscle strength and mass, and are also related to impaired balance and gait, which will in turn increase the risk of falls. Accordingly, this negative spiral of diabetes and ageing will contribute to an enhanced development of disability in activities of daily living and can eventually lead to a quicker loss of independence [14, 15].

In contrast to the well-established findings on maximal muscle strength, little is known about the impact of T2DM and dNP on muscle endurance and explosive muscle strength. As both are crucial muscle function parameters, closely related to activities of daily living and quality of life [15, 16], this flaw in knowledge and insight may be responsible for suboptimal treatment effects of exercise and rehabilitation. Particularly in the elderly, decreased maximal muscle strength, force steadiness (i.e. strength-endurance), and power (i.e. explosive strength) are strongly associated with an increased risk for functional limitations, disabilities, and with higher probability of falls [17]. Fatigability of skeletal muscles can limit the performance of daily tasks that require repeated or sustained contractions [18, 19]. The functional relevance of explosive strength (i.e. rapidly available strength) is mandatory in order to avoid falls and hip fractures in older adults [20, 21].

To our knowledge, only two studies examined muscle endurance in T2DM patients with dNP, and reported reduced levels of lower limb endurance in these patients. These findings were associated with impaired mobility and poor quality of life [16, 22]. Additionally, in a T2DM population with exclusion of clinically suspected dNP, Senefeld et al. (2018) provided pioneering work on the contribution of neural (i.e. (supra)spinal) and muscular (i.e. contractile) mechanisms to a greater fatigability in the knee extensor muscles compared to healthy participants after a dynamic fatiguing task [18]. Yet, the influence of T2DM and dNP on explosive muscle strength remains unexplored.
Currently, it is unclear whether the different measures of muscle strength and muscle mass are differently affected in patients with either dNPs or dNPsm. We hypothesized that both muscle endurance and explosive muscle strength are affected in T2DM patients without and with neuropathy compared to a healthy control group in the same age category. This affection is hypothesized to increment from patients without diabetic neuropathy, over patients with sensory diabetic neuropathy into patients with sensorimotor diabetic neuropathy.

In order to both optimize and customize the recommendations for strength training, the aim of this study was to examine the impact of sensory and sensorimotor dNP on lower limb endurance, explosive and maximal muscle strength, compared to T2DM patients without dNP and healthy controls.

Participants, Materials and Methods

Study design and participants

In this observational comparative study, 35 patients with T2DM and 19 healthy volunteers (C) were included (N=54). Patients were classified into the following groups: T2DM patients without dNP (dNP-; n=8), with sensory dNP (dNPs; n=13) and those with sensorimotor dNP (dNPsm; n=14).

In order to be eligible for this study, participants had to be male, aged between 55 and 85, had to be able to understand Dutch instructions and to walk independently with or without walking aids. Participants were excluded when they experienced (i) neurological conditions (e.g. stroke, dementia, other causes of nerve injury and/or non-diabetic neuropathy), (ii) musculoskeletal disabilities (e.g. foot ulcerations and lower limb amputations), (iii) severe cardiovascular diseases (e.g. chronic heart failure), (iv)

respiratory diseases (chronic obstructive lung diseases), and (v) severe liver dysfunction and/or renal failure.

Patients with T2DM were recruited by endocrinologists at the Department of Endocrinology (Ghent University Hospital) or by general practitioners. T2DM was diagnosed according to the ADA-criteria [23]. Only controls without neuropathy were eligible and incorporated into the study by means of online advertising, flyer distribution, and from acquaintances of the researchers.

The present study was carried out with the approval of the Ethical Committee of the Ghent University Hospital (B670201112900) and all participants provided a written informed consent for participation.

Participant characteristics

Demographic data were gathered during anamnesis. Relevant medical history (e.g. medication and the duration of diabetes) was asked or obtained through medical files.

Anthropometric data

Height and weight were measured and body mass index (BMI) was calculated.

Total-body dual-energy X-ray absorptiometry (DXA) was performed to determine total lean body mass (LBM^{tot}; kg), total fat mass (FM^{tot}; kg) and LBM of the participant 's dominant leg (LBM^{leg}; kg) using a Hologic QDR 4500 DXA Discovery A device (Hologic Inc., Bedford, MA, USA). Peripheral quantitative computed tomography (pQCT; CXT-2000, Stratec Medizintechnik, Pforzheim, Germany) was used to scan the dominant leg (66% of the tibia length) in order to assess muscle density (mg/cm^3).

Blood samples

HbA1c, glucose and lipid profile (total cholesterol, LDL-C, HDL-C and triglycerides) were assessed in fasting venous blood. HbA1c levels were determined using a Menarini HA-8140 analyzer. Glucose was analyzed by the hexokinase method (COBAS, Roche). The lipid variables were evaluated using diagnostic kits (Roche Diagnostics) for HDL-C (cholesterol oxidase-PEG), triglycerides (glycerol phosphate-PAP) and total cholesterol (cholesterol oxidase-PAP). LDL-C was calculated from total cholesterol, HDL-C and triglycerides.

Habitual behavior assessments

The level of physical activity was recorded by the Baecke questionnaire [24]. Smoking habits were recorded as 'currently smoking' or 'ever smoked', and were quantified in packyears. Habitual alcohol drinkers were defined as participants who consumed at least 20 gram of pure alcohol in one day at least three times per week.

Measurements of arterial stiffness, limited joint mobility and neuropathy

The ankle-brachial index was automatically calculated by means of the Microlife WatchBP Office ABI (Microlife®, Florida, USA).
A goniometer was used to detect limited joint mobility with passive range of motion measurements at the dominant knee and ankle.
The presence (and potential type and severity) of NP was determined in all participants by a board-certified neurologist at the Department of Neurology (Ghent University Hospital) using electroneuromyography (CareFusion Nicolet EDX®, Middleton, USA) with synergy software analysis (Version 20.0 EDX®). The electrodiagnostic reference values for these upper and lower limb nerve conduction studies in adult populations were used according to Chen et al. (2016) [25]. The motor nerve conduction of the N. Peroneus communis, N. Tibialis, and N. Ulnaris was evaluated at the most affected limb, indicated by the participant's complaints, and was reported as compound muscle action potential (mV) and motor nerve conduction velocity (m/s). The sensory nerve action potential (µV) and sensory nerve conduction velocity (m/s) were measured using an antidromic technique and consisted of stimulation of the N. Suralis and N. Radialis on both sides of the body (Table A.1). These sensory measurements were obtained while skin temperature, recorded over the dorsum of the hand and foot, was maintained at a minimum of 30°C.

Measurements of maximal muscle strength and muscle endurance

An isometric (IM) and isokinetic (IK) evaluation was performed by using dynamometry (Biodex®; Biodex Corporation, Shirley, NY, USA) in order to measure the maximal voluntary muscle strength of the extensors and flexors of knee and ankle. Test procedures were followed as described in the Biodex Manual and were performed at the dominant leg.

IM and IK maximal peak torque per lean leg mass (PT/LBM^{leg}; Nm/kg) were measured and calculated. All IM assessments were performed twice and lasted for five seconds each, with a resting interval of 60 seconds between the assessments, preceded by two trial tests. For optimal IM functioning, the knee was positioned and fixed at 60° flexion to assess knee extension and at 30° for knee flexion; the reference angle of the ankle was 0°. The concentric and eccentric IK torques were assessed at $60°s^{-1}$ and consisted of five repetitions. The highest value was considered. After a pre-session, the participants were asked to push and pull as hard and fast as possible throughout the full available range of motion with verbal encouragement of the researcher.

IK assessments were also used to measure muscle endurance by means of total work (J) and by calculating the work-fatigue ratio, which is the percentage decrease in torque output between $work^{1/3}$ and $work^{3/3}$, divided by $work^{1/3}$. These concentric and eccentric IK torques were assessed at $180°s^{-1}$ and consisted of 30 repetitions for the knee and 20 for the ankle, and were verbally encouraged by the same researcher.

It is noteworthy that isokinetic endurance dynamometry is a psychophysical test, requiring full cooperation of the participant. Therefore, the evaluations of strength at knee and ankle were obtained with an intra-individual variation (i.e. coefficient of variance) of less than 10% [26].

Measurements of explosive muscle strength and functional performance

In this study, the single two leg jump (s2LJ) test and the chair rising test (CRT), respectively representing explosive muscle strength and functional performance, were carried out according to Taani et al. (2017), in random sequence on the LEONARDO® mechanography ground reaction force platform (NOVOTEC Medical Gmbh, Pforzheim, Germany) [27]. In both tests, peak force (N), power/BW (W/kg), and maximal velocity (m/s) were calculated. Additionally, maximal height (m) was estimated in the s2LJ test. The Esslinger Fitness Index, calculated in both s2LJ and CRT, represents the maximal jump power relative to BW for one's age- and gender-matched reference population [28].

Statistical analysis

Data were analyzed using IBM Statistical Package for Social Sciences (SPSS version 25) and an alpha level of 0.05 (two-tailed) was used. The approximate normality of data was examined by the Shapiro–Wilk test. Descriptive statistics for anthropometric,

biochemical and muscle parameters are presented as mean (± SD) unless otherwise stated. Participant's characteristics were analyzed with a univariate ANOVA to compare subgroups.

A Pearson Chi-Square test was used for smoking habits, ethyl consumption and use of medication in order to detect all between-group differences.

Between-groups analysis of knee and ankle endurance was performed using ANCOVA with LBM^{leg} as covariate. Explosive muscle strength and functional performance outcomes (total force, velocity, estimated height, and the Esslinger Fitness Index) were analyzed by means of ANCOVA with total BW as covariate. Relative total power (corrected for total BW) and relative maximal muscle strength (corrected for LBM^{leg}) were analyzed using ANOVA. Post hoc comparisons were performed by means of the Sidak test.

Results

Participant characteristics

Table 1 reports on general and clinical participant characteristics. Age and habitual behavior assessments (level of physical activity, smoking habits, and alcohol consumption) were not different between healthy controls and the subgroups of T2DM patients. Also, anthropometric characteristics (BMI, LBM^{tot}, LBM^{leg}, and FM^{tot}) were not significantly different between the different groups. Only leg muscle density (pQCT) showed a tendency towards significance between healthy controls and dNPsm (p=0.051).

In the overall patient group, diabetes duration ranged from 2 to 31 years with a mean of 13 years with an average HbA1c of 7.4% (±1.03). All patients used oral anti-diabetes medication and/or insulin. In the healthy control group, the average HbA1c level was ≤6.0% without intake of glucose-lowering medication (Table A.2).

No significant differences between healthy controls and the diabetes groups were found in both ankle and knee range of motion and in the ankle-brachial index.

Table 1: General and clinical participant characteristics

	C (n=19)	**dNP-** (n=8)	**dNPs** (n=13)	**dNPsm** (n=14)
Age *(years)*	64 (6.7)	65 (3.2)	66 (6.9)	67 (8.3)
BMI *(kg/m^2)*	27 (3.3)	29 (3.5)	29 (6.0)	31 (4.0)
Body height *(m)*	1.75 (0.066)	1.76 (0.529)	1.77 (0.063)	1.76 (0.064)
Body weight *(kg)*	82.6 (11.18)	91.2 (14.72)	90.2 (18.88)	95.2 (12.72)
LBM^{tot} *(kg)*	61.5 (6.9)	66.1 (9.57)	65.7 (10.01)	68.7 (8.41)
LBM^{leg} *(kg)*	9.6 (1.10)	10.0 (1.50)	9.8 (1.49)	10.1 (1.50)
FM^{tot} *(kg)*	18.5 (5.00)	22.3 (5.80)	21.6 (10.00)	23.4 (6.47)
Leg muscle density (*mg/cm^3*)	73.3 (3.76)	72.9 (3.81)	72.5 (4.02)	69.2 (5.10)
Level of PA (/15)	8.0 (6.25-9.63)	8.5 (5.50-9.50)	8.00 (5.13-10.13)	7.6 (6.63-10.25)
Smoking habits:				
currently smoking (*%)*	33.3	16.7	41.7	25.0
ever smoked (*%)*	88.9	66.7	66.7	91.7
Packyears (*n*)	16.9 (16.20)	18.0 (21.16)	14.1 (16.81)	14.9 (18.39)
Habitual alcohol drinkers (%)	44.4	12.5	23.1	21.4
Diabetes duration *(years)*	NA	10 (7.8)	13 (6.8)	15 (9.6)
HbA1c *(%)*	5.6 (0.22)	7.4 (0.84)*	6.9 (0.58)*	7.8 (1.29)*
(mmol/mol)	38.3 (2.36)	57.0 (9.10)*	51.9 (6.25)*	61.7 (14.13)*
Glucose *(mg/dL)*	99.5 (13.19)	147.4 (39.59)	195.8 (153.78)	176.0 (58.66)
Cholesterol total *(mg/dL)*	200.4 (46.49)	164.6 (27.07)	163.4 (38.97)	168.2 (48.10)
LDL *(mg/dL)*	112.4 (40.48)	85.6 (21.67)	89.5 (31.41)	79.9 (37.00)
HDL *(mg/dL)*	63.3 (18.24)	58.1 (15.65)	51.6 (14.12)	54.8 (31.82)
Triglycerides *(mg/dL)*	120.6 (67.78)	102.0 (32.11)	110.9 (43.98)	193.9 (225.68)
RoM knee *(°)*	131 (119-140)	140 (125-140)	135.5 (110-154)	130 (50-140)
ankle *(°)*	60 (37-88)	52 (50-90)	59 (35-70)	52 (23-78)
ABI *(ratio)*	1.3 (0.15)	1.3 (0.06)	1.2 (0.20)	1.2 (0.17)

Data are expressed as mean (SD), with exception for the level of PA (n=39) and RoM, both expressed as median (min-max). The percentages of participants with a history of smoking and ethyl consumption are presented.

C, healthy controls without neuropathy; dNP-, patients without diabetic neuropathy (dNP); dNPs, patients with sensory dNP; dNPsm, patients with sensorimotor dNP; BMI, body mass index; LBM^{tot}, total lean body mass; LBM^{leg}, lean body mass of the dominant leg; FM^{tot}, total fat mass; PA, physical activity; RoM, range of motion; ABI, ankle-brachial index; NA, not applicable.

***** $p<0.05$ compared to C

Muscle endurance in the distal lower dominant limb

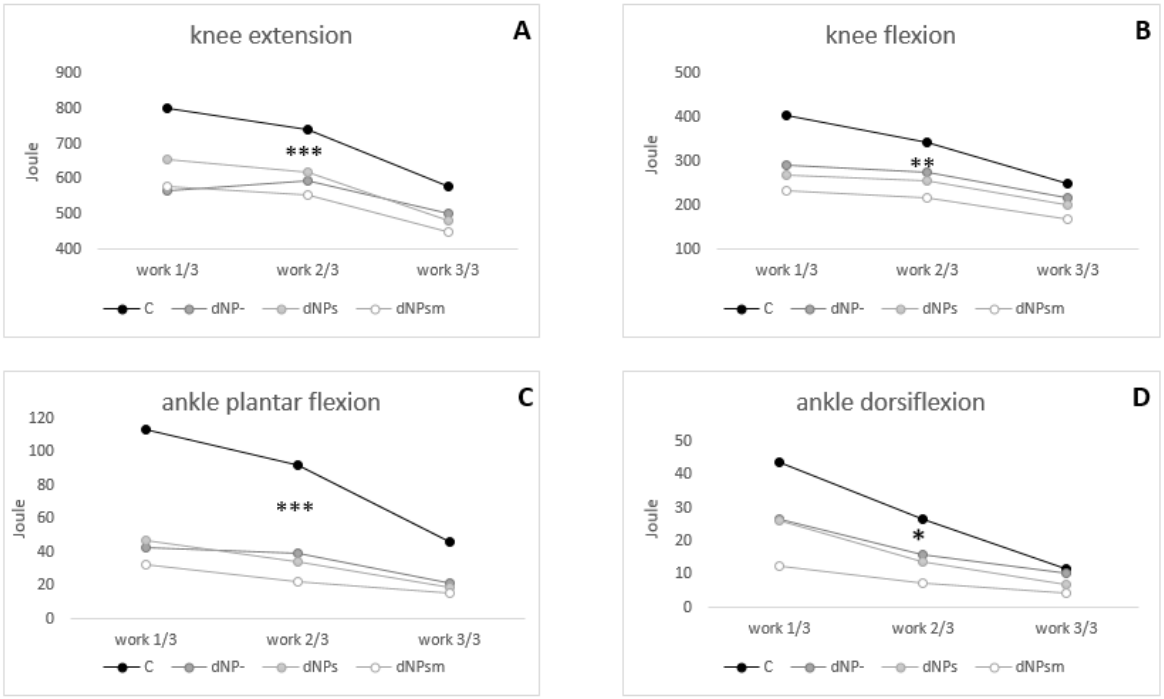
Work$^{1/3}$, work$^{2/3}$, work$^{3/3}$ and total work (J), expressed as area under the curve, are presented in Figure 1 (A-D). Additionally, Table A.3 presents an overview of the raw data.

Total work in IK knee extension and ankle plantar flexion was higher for the healthy controls compared to dNP- (p=0.023 and p=0.002), dNPs (p=0.043 and p=0.001), and dNPsm (p=0.004 and p=0.000).

Furthermore, the healthy controls scored significantly higher in total work knee flexion compared to dNPs (p=0.011) and dNPsm (p=0.001), and in total work ankle dorsiflexion compared to dNPsm only (p=0.000).

The work-fatigue ratio did not reveal significant differences between the four groups.

Figure 1: Absolute IK muscle endurance expressed in work 1/3, work 2/3, and work 3/3 in (A) knee extension, (B) knee flexion, (C) ankle plantar flexion, and (D) ankle dorsiflexion



IK, isokinetic; C, healthy controls without neuropathy; dNP-, patients without diabetic neuropathy (dNP); dNPs, patients with sensory dNP; dNPsm, patients with sensorimotor dNP.
* p<0.05 for total work (area under the curve) in C versus dNPsm
** p<0.05 for total work (area under the curve) in C versus dNPs, and in C versus dNPsm
*** p<0.05 for total work (area under the curve) in C versus dNP-, in C versus dNPs, and in C versus dNPsm

Explosive muscle strength and functional performance

Both total power and the Esslinger Fitness Index of the single two leg jump test were significantly higher in the healthy controls compared to dNPsm (p=0.004 and p=0.020). Also, a tendency towards significance between the healthy controls and dNPsm was observed for velocity and estimated maximal height (p=0.057 and p=0.079).
No significant differences were found between the four groups neither for the single two leg jump total force, nor for functional performance (chair rising test) (Table 2).

Table 2: Explosive muscle strength and functional performance

	C (n=19)	**dNP-** (n=8)	**dNPs** (n=13)	**dNPsm** (n=14)
Explosive muscle strength: s2LJ test				
total force max *(kN)*	1.88 (0.370)	1.85 (0.427)	1.83 (0.403)	1.97 (0.222)
total power max/BW *(W/kg)*	38.11 (6.303)	28.79 (7.044)	29.81 (5.234)	26.65 (8.124)*
velocity max *(m/s)*	2.16 (0.192)	1.82 (0.300)	1.83 (0.261)	1.71 (0.445)
estimated height max *(m)*	0,33 (0,038)	0,24 (0,068)	0,25 (0,058)	0,23 (0,106)
E.F.I. *(%)*	98.4 (13.73)	77.3 (17.37)	82.4 (18.11)	73.6 (19.47)*
Functional performance: CRT				
total force max *(kN)*	1.16 (0.135)	1.25 (0.173)	1.22 (0.246)	1.27 (0.181)
total power max/BW *(W/kg)*	11.41 (3.846)	8.87 (2.074)	9.01 (1.483)	9.09 (2.438)
velocity max *(m/s)*	1.10 (0.251)	0.95 (0.138)	0.91 (0.126)	0.90 (0.174)
E.F.I. *(%)*	101.3 (12.57)	90.0 (14.96)	93.2 (21.62)	84.1 (20.18)

All data are expressed as mean (SD).

C, healthy controls without neuropathy; dNP-, patients without diabetic neuropathy (dNP); dNPs, patients with sensory dNP; dNPsm, patients with sensorimotor dNP; s2LJ, single two leg jump; BW, body weight; E.F.I., Esslinger Fitness Index; CRT, chair rising test.

***** p<0.05 compared to C

Relative maximal muscle strength in the distal lower dominant limb

Table 3 reports on relative IM and IK maximal knee extension and flexion muscle strength, with only significantly higher IK maximal knee flexion muscle strength in the healthy controls compared to dNPsm (p=0.013). Considering the ankle, significantly better results for IK maximal plantar flexion muscle strength were found in the healthy controls compared to dNPsm (p=0.002).

Relative IM and IK maximal dorsiflexion muscle strength revealed significantly higher values in the healthy controls compared to dNPsm (p=0.035 and p=0.003). Additionally, relative IM and IK dorsiflexion strength values were better in dNP- compared to dNPsm (p=0.021 and a tendency towards significance (p=0.085) respectively).

Table 3: Relative maximal muscle strength of the lower limb (dominant knee and ankle)

	C (n=19)	dNP- (n=8)	dNPs (n=13)	dNPsm (n=14)
Knee extension				
IM max PT/LBM^{leg} *(Nm/kg)*	15.5 (3.05)	13.6 (3.15)	12.9 (2.94)	13.1 (3.69)
IK max PT/LBM^{leg} *(Nm/kg)*	14.3 (3.44)	11.9 (2.86)	12.6 (2.50)	11.7 (3.26)
Knee flexion				
IM max PT/LBM^{leg} *(Nm/kg)*	10.5 (1.58)	8.5 (1.40)	8.7 (2.49)	8.9 (2.25)
IK max P/LBM^{leg} *(Nm/kg)*	7.4 (1.36)	6.5 (0.91)	6.3 (1.21)	5.8 (1.73)*
Ankle plantar flexion				
IM max PT/LBM^{leg} *(Nm/kg)*	9.2 (2.70)	6.6 (3.01)	7.5 (1.64)	6.8 (2.57)
IK max PT/LBM^{leg} *(Nm/kg)*	5.8 (2.04)	5.0 (2.96)	3.5 (1.24)	2.6 (1.45)*
Ankle dorsiflexion				
IM max PT/LBM^{leg} *(Nm/kg)*	3.3 (1.17)	3.5 (1.06)	2.7 (1.08)	2.0 (0.71)*†
IK max PT/LBM^{leg} *(Nm/kg)*	2.4 (0.70)	2.2 (0.50)	2.0 (0.53)	1.5 (0.41)*

All data are expressed as mean (SD).

C, healthy controls without neuropathy; dNP-, patients without diabetic neuropathy (dNP); dNPs, patients with sensory dNP; dNPsm, patients with sensorimotor dNP; IM, isometric; PT, peak torque; LBM^{leg}, lean body mass of the dominant leg; IK, isokinetic.

***** p<0.05 compared to C

† p<0.05 compared to dNP-

Discussion

Main findings

In this study, a reduction in lower limb endurance was demonstrated by means of lower levels for total work in knee extension/flexion (20-30%) and ankle PF/DF (50-60%) in patients with T2DM (with or without dNP) compared to healthy controls. No noticeable differences in the work-fatigue ratio between all study groups were found. Both explosive and maximal muscle strength were significantly reduced in dNPsm compared to healthy controls.

The results of this study indicate a deteriorating effect on explosive and maximal muscle strength due to the presence of sensorimotor neuropathy, while T2DM as such predominantly affects muscle endurance.

Muscle endurance

Our findings on lower limb fatigability are in line with the scarce literature using comparable muscle endurance protocols. Allen et al. (2015) found a significant reduction in the average time to exhaustion between T2DM patients with dNP (dNP+) and age- and gender-matched healthy controls [22]. IJzerman et al. (2012) reported no significant differences in work-fatigue ratio based on the index of Moreau et al. [29], comparing dNP-, dNP+ and healthy controls, except for the knee flexor outcome in dNP- versus healthy controls [16]. Additionally, Senefeld et al. (2018), compared patients with T2DM without clinical signs of dNP to age-, BMI- and physical activity-matched healthy controls, revealing a greater fatigability of the patient's knee extensor muscles. The greater fatigability was primarily associated with an impaired glycemic control and altered contractile mechanisms [18]. Bearing in mind that in previous research no further distinction was made between the type and severity of dNP, our results may give more in-depth information on the impact of dNP on muscle strength.

The absence of a compelling difference in lower limb endurance between patients with and without dNP, together with the lower values in our T2DM cohort in general, may indicate that the T2DM pathogenesis rather than the presence of dNP affects muscle endurance. A possible explanation for this discrepancy between healthy participants and patients with T2DM could be the impact of chronic hyperglycemia on the ageing process of skeletal muscle fibers. Indeed, there is a large body of evidence showing

an age-related muscle fiber type shift towards a higher proportion of type I fibers [30, 31]. Interestingly, opposite findings have been demonstrated in the T2DM population with a shift towards a higher proportion of type II muscle fibers [16, 32-35]. This could explain the decreased muscle endurance in patients with T2DM, knowing that the slow-twitch oxidative muscle fibers (type I) are predominantly activated by endurance stimuli. The consequent weakness in the diabetic muscles may be induced by altered cellular metabolism (e.g. insulin resistance, metabolic inflexibility, reduced mitochondrial function, accelerated advanced glycation end products, …) and contractile mechanisms. This may result in an age-related decline in muscle strength prior to the reduction of muscle mass, predominantly pronounced in elderly patients with T2DM [18, 36, 37].

Explosive and maximal muscle strength

Although muscle endurance was reduced in all patients with T2DM, a different pattern was identified with regard to both explosive and maximal muscle strength. Actually, both strength parameters were mainly influenced by the presence of dNPsm, suggesting a more dominant impact of neuropathic disturbances.

Our results concerning maximal muscle strength are in line with previous findings, claiming that the presence of dNP is associated with reduced maximal muscle strength values in patients with T2DM [3-13, 22]. However, no distinction was made between dNPs and dNPsm in these publications. Due to the fact that explosive muscle strength is strongly determined by maximal muscle strength [38], reduced maximal muscle strength in the lower limbs may have influenced the outcome on the s2LJ test.

Explosive and maximal muscle strength are influenced by various neural and morphological mechanisms, with motor unit loss or axonal loss as a feature of dNP. Subclinical motor involvement is often detected on electrophysiological studies in non-diabetic patients with idiopathic sensory polyneuropathy [39]. According to Gutierrez et al. (2001), the presence of mild diabetic neuropathy leads to a decrease in the rapidly available ankle strength in the frontal plane and to a distal impairment in lower limb sensory function, which increases fall risk [21].

Allen et al. (2014) observed that this motor unit loss is accompanied by a loss of muscle strength and mass [12]. Furthermore, the same researchers reported that the muscle weakness in patients with dNP is related to the severity of neuropathy, which provides

an explanation for our finding that patients with dNPsm have the lowest levels of maximal muscle strength. In our study, a significant reduction of maximal muscle strength was also observed in patients with dNPsm for knee flexors as well as for ankle plantar and dorsiflexors. Concerning muscle mass, we could not observe differences in LBM^{leg} (DXA).

Additionally, muscle torque or force is also influenced by muscle density (pQCT), indicating fat infiltration in the muscle [40]. Goodpaster et al. (2001) showed that reduced quadriceps muscle density accounted for differences in maximal muscle torque, which is not attributed to muscle mass [41]. Besides, Allen et al. (2014) showed that a greater loss of motor units (e.g. caused by denervation of muscle fibers) is associated with greater proportions of non-contractile intramuscular tissue (fat and/or connective tissue) and with a proportional loss of contractile tissue. This process will impact the muscle quality negatively (reduced strength per unit muscle mass) [12, 42].

In this study, pQCT data revealed a decreased leg muscle density outcome in the dNPsm group versus healthy controls with a tendency towards significance (p=0.051), indicating an increased fat infiltration in this subgroup, and most likely resulting in poor muscle quality.

A critical note has to be mentioned concerning muscle mechanography. First, the s2LJ test is a very complex coordination and balance task, demanding from the participant peak torque strength performances rather from the large proximal muscle groups (e.g. the Quadriceps and hamstrings), than from the ankle plantar and dorsiflexors. Bearing in mind that the dNPsm group predominantly suffers from reduced maximal muscle strength in the distal lower limbs, the results of this test have to be evaluated with caution. Second, CRT is most commonly used for geriatric purposes as it determines whether elderly meet the minimum criteria for activities of daily living [43]. Consequently, caution is also needed when interpreting these unimpaired results, as activities-specific balance, confidence and diverse performance skills, such as core stability, determine the performance of this functional test [27, 44, 45].

Practical implications

Exercise training is a keystone intervention in patients with T2DM (besides pharmacological and dietary interventions [37]), in order to maintain quality of life and to reduce risk of falls [15, 46]. Based on our results, it can be suggested to target strength training programs depending on the presence or absence of neuropathy in patients with T2DM. While endurance and strength training should generally be recommended in patients with T2DM, muscle strength training programs with high intensities, whether or not combined with higher velocities (power training), seem to be of crucial importance in patients with dNPsm in order to preserve both explosive and maximal muscle strength [47].

Strengths and limitations

In the present study, electroneuromyographic examinations were performed in order to allocate each patient with T2DM to either the dNP- group or dNPs and dNPsm, since this is the gold standard for the diagnosis of neuropathy.

This is the first study that evaluates three major domains of muscle strength (endurance, explosive and maximal) in patients with dNPs and dNPsm, in comparison to dNP- and healthy controls.

Due to the nature of this cross-sectional study design, the future challenge is to establish adequately powered longitudinal research to determine the influence of ageing, the impact of long-term hyperglycemia exposure and the effect of lifestyle adjustments such as physical exercise on muscle strength in T2DM patients without dNP and in those with dNPs or dNPsm compared to healthy controls.

All participants were asked to report their medication intake. Patients with T2DM using confounding medication that could limit functional testing due to an affected neuromuscular status were not excluded. However, notwithstanding the larger heterogeneity of the groups, this limitation results in a sample size that is more representative for the Flemish population with T2DM.

The limited amount of differences between dNP- and patients with more severe neuropathy (dNPs and dNPsm) may be explained by the low number of patients in dNP- (n=8), jeopardizing the power of our results. However, a posteriori power analysis of the primary outcomes (i.e. muscle endurance and explosive muscle strength)

between the four groups (i.e. C, dNP-, dNPs, and dNPsm) exceeds 80% for total work ankle PF/DF and for the s2LJ test.
Generally, muscle strength can also be influenced by a variety of factors, including vitamin D and musculoskeletal pain. Unfortunately, we did not assess these features in this research.

Conclusion

In conclusion, this study presents evidence that diabetes has a significant degrading impact on muscle endurance, while explosive and relative maximal muscle strength were influenced by sensorimotor diabetic neuropathy.

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B.V.E., D.C. and P.C. conceived and designed the study, analyzed data, wrote, edited, and reviewed the manuscript. B.V.E., B.L., P.P., K.V.W. and B.C. researched data, contributed to the discussion and interpretation of data, and edited and reviewed the manuscript. All authors gave final approval for publication. B.V.E. and P.C. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
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Conflicts/disclosure

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

Appendix Table A.1: Electroneuromyography data

		C (n=19)	dNP- (n=8)	dNPs (n=13)	dNPsm (n=14)
SNCV	N. Suralis *(m/s)*	46.0 (6.56)	41.3 (7.48)	33.4 (23.67)	21.0 (31.84)
	N. Radialis	41.6 (5.21)	46.5 (8.93)	41.8 (3.74)	38.1 (5.48)
SNAP	N. Suralis *(μV)*	5.2 (3.09)	7.3 (3.78)	3.2 (3.37)†	0.4 (0.83)*†
	N. Radialis	10.3 (4.30)	17.6 (14.70)	12.5 (6.01)	9.8 (2.73)
MNCV	N. Tibialis *(m/s)*	43.3 (3.47)	42.0 (4.53)	40.1 (5.80)	36.2 (2.82)*
	N. Ulnaris	57.0 (3.38)	59.0 (3.75)	52.9 (6.07)	48.9 (5.08)*†
	N. Peroneus	49.9 (7.84)	49.0 (5.25)	46.6 (7.48)	47.1 (15.10)
CMAP	N. Tibialis *(mV)*	9.4 (4.50)	8.6 (1.94)	5.7 (2.57)	2.9 (2.69)*
	N. Ulnaris	8.7 (2.24)	7.6 (1.16)	7.5 (1.63)	5.1 (2.23)*
	N. Peroneus	5.6 (1.51)	6.8 (2.06)	4.9 (1.44)†	3.5 (2.50)

All data are expressed as mean (SD).

C, healthy controls without neuropathy; dNP-, patients without diabetic neuropathy (dNP); dNPs, patients with sensory dNP; dNPsm, patients with sensorimotor dNP; SNCV, sensory nerve conduction velocity; SNAP, sensory nerve action potential; MNCV, motor nerve conduction velocity; CMAP, compound motor action potential; N., nervus.

***** $p<0.05$ compared to C

† $p<0.05$ compared to dNP-

Appendix Table A.2: Medication

	C (n=19)	dNP- (n=8)	dNPs (n=13)	dNPsm (n=14)
DM medication oral *(%)*	0	100	84.6	71.4
Metformin® *(%)*	0	62.5	69.2	42.9
Januvia® *(%)*	0	12.5	0	7.1
DM insulin injection *(%)*	0	37.5	50.0	85.7
Lantus® *(%)*	0	0	23.1	28.6
Humalog® *(%)*	0	12.5	0	7.1
Novorapid® *(%)*	0	12.5	15.4	14.3
Other medication *(%)*	57.9	87.5	69.2	78.6
NSAIDs *(%)*	0	12.5	0	0
Anticoagulants *(%)*	15.8	50.0	46.2	71.4
Cholesterol-lowering *(%)*	31.6	75.0	23.1	57.1
Antihypertensive *(%)*	26.3	62.5	53.8	71.4

The percentages of each participant's relevant medication intake are presented.

C, healthy controls without neuropathy; dNP-, patients without diabetic neuropathy (dNP); dNPs, patients with sensory dNP; dNPsm, patients with sensorimotor dNP; DM, diabetes mellitus; NSAIDs, nonsteroidal anti-inflammatory drugs.

Appendix Table A.3: Muscle endurance of the lower limb (dominant knee and ankle)

	C (n=19)	dNP- (n=8)	dNPs (n=13)	dNPsm (n=14)
IK knee extension				
total work *(J)*	2124.8 (480.33)	1667.6 (141.05)*	1761.3 (480.04)*	1583.6 (681.77)*
work-fatigue (%)	25.6 (13.35)	4.3 (41.91)	25.6 (16.20)	17.9 (17.73)
work$^{1/3}$ *(J)*	803.3 (214.87)	568.7 (119.53)*	656.6 (167.36)	578.0 (265.65)*
work$^{3/3}$ *(J)*	579.3 (121.08)	502.6 (114.65)	483.2 (153.46)*	448.4 (184.46)*
IK knee flexion				
total work *(J)*	998.9 (291.43)	787.3 (234.22)	728.0 (316.33)*	622.6 (375.91)*
work-fatigue (%)	37.9 (21.39)	24.5 (38.99)	23.3 (29.41)	27.9 (27.53)
work$^{1/3}$ *(J)*	406.5 (125.23)	292.9 (89.05)*	269.2 (107.78)*	233.6 (151.31)*
work$^{3/3}$ *(J)*	248.6 (89.04)	217.5 (101.63)	202.2 (107.91)	170.1 (99.09)
IK ankle plantar flexion				
total work *(J)*	252.6 (80.27)	103.9 (55.32)*	100.4 (60.12)*	70.3 (92.84)*
work-fatigue (%)	56.0 (15.97)	61.2 (22.31)	62.0 (37.71)	67.3 (24.18)
work$^{1/3}$ *(J)*	113.5 (40.98)	42.9 (26.76)*	46.8 (20.25)*	32.9 (39.56)*
work$^{3/3}$ *(J)*	46.6 (14.72)	21.2 (18.15)*	19.1 (19.64)*	15.2 (20.97)*
IK ankle dorsiflexion				
total work *(J)*	82.0 (26.02)	52.5 (45.57)	47.0 (44.81)	24.1 (29.74)*
work-fatigue (%)	71.9 (28.35)	71.9 (36.29)	81.9 (30.91)	51.1 (47.62)
work$^{1/3}$ *(J)*	43.5 (11.57)	26.6 (12.70)*	26.3 (17.61)*	12.6 (12.99)*†‡
work$^{3/3}$ *(J)*	11.8 (11.30)	10.1 (15.32)	6.8 (12.96)	4.1 (7.82)

All data are expressed as mean (SD).

C, healthy controls without neuropathy; dNP-, patients without diabetic neuropathy (dNP); dNPs, patients with sensory dNP; dNPsm, patients with sensorimotor dNP.

***** $p<0.05$ compared to C

† $p<0.05$ compared to dNP-

‡ $p<0.05$ compared to dNPs

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3.

The impact of diabetic neuropathy on the distal versus proximal comparison of weakness in lower and upper limb muscles of patients with type 2 diabetes mellitus: a cross-sectional study

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Abstract

Objectives: This study aimed to determine the impact of diabetic neuropathy (dNP) on the distal versus proximal comparison of weakness in lower and upper limb muscles of patients with type 2 Diabetes Mellitus (T2DM).

Methods: 19 healthy male controls without neuropathy (HC) and 35 male T2DM patients, without dNP (n=8), with sensory dNP (n=13) or with sensorimotor dNP (dNPsm; n=14), were enrolled in this study. Maximal isometric (IM) and isokinetic (IK) muscle strength and IK muscle endurance of the dominant knee, ankle and elbow, and maximal IM handgrip strength were measured by means of dynamometry.

Results: Ankle muscle endurance was lower compared to the knee, independently of dNP ($p<0.001$). Maximal IK ankle muscle strength was also lower compared to the knee, albeit only in dNPsm ($p=0.003$). No differences were found between maximal IM handgrip and elbow strength.

Conclusions: Our results suggest an impact of T2DM -with or without dNP- on lower limb muscle strength more distally than proximally, while this was not observed in the upper limb. The gradient of dNP seemed to be a determining factor for the maximal muscle strength, and not for muscle endurance, in the lower limb.

Key Words: Diabetic Neuropathy, Dstal Versus Proximal Comparison, Lower And Upper Limb, Muscle Strength, Type 2 Diabetes Mellitus

Introduction

Diabetic neuropathy (dNP) affects approximately 50% of the patients with type 2 diabetes mellitus (T2DM). This complication accelerates age-related declines in muscle strength and muscle mass and contributes to an increased risk of falls and the development of difficulties in mobility and functionality. Accordingly, dNP can have a paramount impact on daily life activities and is associated with loss of independence and a reduced quality of life [1-7].

This neuropathic disorder most often affects the sensory nerves. Thus, initially, sensory dNP (dNPs) presents with sensory disturbances such as neuropathic pain and a decreased sense of vibration, temperature and light touch. At a later stage, also sensorimotor dNP (dNPsm) may develop with motor disturbances such as skeletal muscle weakness and atrophy [3, 8]. These symptoms can be observed in the lower limbs, starting at the ankles and usually progressing in a distal-to-proximal way towards the knee [9].

The T2DM disease itself [10-17], but also the presence of dNP [5, 18-25], contributes to the deterioration of *maximal muscle strength* in the ankle and knee joints compared to healthy controls (HC). In contrast to the extensive knowledge on maximal muscle strength in patients with T2DM, available data on *muscle endurance* and *explosive strength* or *power* in this population are scarce [5, 11]. In 2020, our research group already reported a negative impact of the presence of dNPsm on maximal muscle strength and explosive strength, but not on muscle endurance, which was only affected by the T2DM disease as such [26].

The first symptoms of dNP become manifest at the lower limbs. However, symptoms at the upper limbs can develop as well, especially when dNP is present for at least 20 years [3, 27]. In 2014, it was reported that muscle weakness in the hand may occur the moment dNP advances from the ankle to the level of the knee [3]. To our knowledge, a comparison of muscle weakness in the distal (hands) versus proximal (elbow) part of the upper limb, eventually due to the length-dependent nature of dNP, has not been discussed in literature.

Furthermore, the impact of sensory and motor impairments on functional domains such as activities of daily living and self-care may be more significant in the upper limb

compared to the lower limb [28]. However, no data were found on the association between dNP and the physical performance of the upper limb.

Based on these knowledge gaps in literature, the aim of this study was to determine whether muscle weakness was more pronounced in the distal (ankle) compared to proximal (knee) part of the lower limb and in the distal (hands) compared to proximal (elbow) part of the upper limb in patients with T2DM and, if present, whether this was affected by the presence and severity of dNP.

Materials and methods

Participants

This observational comparative study presents data of 35 patients with T2DM and 19 healthy controls (HC). Inclusion criteria comprised male gender, aged between 55 and 85 years, able to adequately respond to instructions and to walk independently with or without walking aids.

Participants with the following conditions were excluded: (i) major neurological conditions (stroke, Parkinson's disease, dementia, other causes of nerve injury and/or non-diabetic neuropathy, e.g. radiculopathies), (ii) musculoskeletal disabilities (e.g. upper and lower extremity ulcerations and/or amputations), (iii) severe cardiovascular diseases (e.g. chronic heart failure), (iv) respiratory diseases (chronic obstructive lung diseases), and (v) severe liver dysfunction and/or renal failure.

Patients with T2DM were recruited at the Department of Endocrinology of Ghent University Hospital or by their general practitioner. T2DM was diagnosed in accordance with criteria established by the American Diabetes Association [29]. HC were recruited by online advertising and flyer distribution, and from acquaintances of the researchers. The HCs were only eligible to participate when neuropathy was diagnostically excluded, based on electroneuromyography (ENMG) performed by an experienced specialist at the Department of Neurology of Ghent University Hospital.

The present study was carried out with the approval of the Ethical Committee of Ghent University Hospital (B670201112900), according to the World Medical Association Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. All participants provided a written informed consent for participation.

Participants characteristics

Demographic data were gathered by anamnesis, and the medical history (e.g. medication and the duration of diabetes) was asked or obtained through medical records.

Anthropometric data and body composition

Body height and weight were measured, and the body mass index (BMI) was calculated. Body composition was measured by total-body dual-energy X-ray absorptiometry (DXA). Total fat mass (FM^{tot}; kg), and total lean body mass (LBM^{tot}; kg), LBM of the subject's dominant arm (LBM^{arm}; kg) and leg (LBM^{leg}; kg) were determined using a Hologic QDR 4500 DXA Discovery A device (Hologic Inc., Bedford, MA, USA) [30].

Fasting venous blood samples

HbA1c, glucose, and lipid profile (total cholesterol, LDL-C, HDL-C and triglycerides) were determined. HbA1c was determined using a Menarini HA-8140 analyzer. Glucose was analyzed by the hexokinase method (COBAS, Roche). The lipid variables were evaluated using diagnostic kits (Roche Diagnostics) for HDL-C, triglycerides and total cholesterol. LDL-C was calculated from total cholesterol and HDL-C [26].

Habitual behavior assessments

The level of physical activity was recorded by the Baecke questionnaire, a short survey on activities of daily living [31]. Smoking habits were recorded as 'currently smoking', 'ever smoked' or 'never smoked', and were quantified in pack years [26]. Habitual alcohol drinkers were identified when the alcohol consumption exceeded 20g of pure alcohol in one day at least three days per week [32].

Measurements of neuropathy

Each participant underwent an electrophysiological examination at the most affected limb indicated by the participant in order to determine the presence (and potential type and severity) of dNP. This ENMG was performed by a board-certified specialist, who was blinded for the physical examinations. This procedure has been comprehensively described elsewhere 26. Based on this method, patients were allocated to a group

without dNP (dNP-; n=8), a group with sensory dNP (dNPs; n=13) or a group with sensorimotor dNP (dNPsm; n=14).

Measurements of muscle strength

The extensors and flexors of elbow, knee and ankle joints were measured by means of an isometric (IM) and isokinetic (IK) maximal voluntary muscle strength test battery on the Biodex® dynamometer (Biodex® Corporation). The protocol as described in the Biodex® manual was used and measurements were performed at the dominant upper and lower limb [33]. Data are reported as absolute value and as maximal elbow peak torque per lean arm mass (PT/LBM^{arm}; Nm/kg), and knee and ankle peak torque per lean leg mass (PT/LBM^{leg}; Nm/kg).

All IM assessments were performed twice and lasted for five seconds each, with a resting interval of 60 seconds between consecutive assessments, preceded by two trial tests. For optimal IM functioning, the elbow was positioned and fixed at 90° flexion, the knee at 60° flexion to assess knee extension and at 30° for knee flexion, the reference angle of the ankle was 0° [33].

The concentric and eccentric IK torques were assessed at $60°.s^{-1}$ and consisted of five repetitions. The highest value was considered. After one trial test, the participants were asked to push and pull as hard and fast as possible over the full range of motion with verbal encouragement of the researcher.

IK assessments at the elbow, knee and ankle joints were performed as well to measure muscle endurance. Data are reported as total work (J). Muscle endurance was assessed at $180°.s^{-1}$, consisted of 25, 30 and 20 repetitions for elbow, knee and ankle in respective order, and were all verbally encouraged by the same researcher.

The handgrip strength (HGS; kg) was measured isometrically at the dominant side using the Jamar® dynamometer (Sammsons Preston Rolyan Inc.), according to the American Society of Hand Therapists guidelines [34-36]. A 15-second interval was used between consecutive measurements and the strongest of three attempts was retained as maximal grip strength [37].

Data management and statistical analysis

Data were analyzed using IBM Statistical Package for Social Sciences (SPSS version 26) and an alpha level of 0.05 was used. The normality of data was examined by Q-Q

plots and by the Shapiro-Wilk test. Descriptive data are presented as mean and standard deviations (±SD) unless otherwise stated. Subject characteristics were analyzed with a univariate analysis of variance, i.e. one-way ANOVA with post-hoc Sidak. A Pearson Chi-Square test was used to compare alcohol consumption and smoking habits between the four groups [26].

The raw data of lower limb were already published in a previous article by Van Eetvelde et al, 2020 [26]. For the purpose of this publication, other statistical analyses on upper and lower limb strength were implemented.

For distal versus proximal comparison, the summation of knee flexion and extension (IM and IK maximal peak torque, and IK total work separately) was compared to the summation of ankle plantar flexion (PF) and dorsiflexion (DF). The summation of elbow flexion and extension (IM and IK maximal peak torque, and total work separately) was compared to the summation of knee flexion and extension to analyze whether upper and lower limbs were differently affected. Then, the authors followed the analytical approach of Gosselinck et al., who expressed respiratory and peripheral muscle strength of 44 patients with COPD as a percentage of the control subjects' value (% control) (HC; n=22) with the difference that Gosselink et al. could rely on normalized values (expressed as a percentage of predicted value) of the in- and expiratory muscle strength in healthy, age-, weight-, and gender-matched controls, while no normalized values of peripheral muscle strength are available [38]. So, ratios of each of the three T2DM groups to the HC were calculated for relative maximal muscle strength in hand, elbow, knee and ankle and for muscle endurance in elbow, knee and ankle. These ratios were calculated by subtracting the mean value of the control group (HC^m) from the individual strength value of the diabetic group (dNP^i), divided by the mean value of the control group, i.e. ($dNP\text{-}^i - HC^m$)/HC^m, ($dNPs^i - HC^m$)/HC^m and ($dNPsm^i - HC^m$)/ HC^m.

Repeated measures ANOVA was carried out to detect (i) significant differences between groups (dNP-, dNPs, and dNPsm ratios) within a specific joint (hand, elbow, knee or ankle), (ii) and significant differences within groups (dNP-, dNPs, or dNPsm ratios) between two joints of our interest (e.g. knee versus ankle for distal-proximal evaluation, elbow versus knee for upper-lower evaluation, …). For this test, the level of significance was set at $p<0.05$. When significant differences were found for (i) a post-hoc analysis was performed with an independent sample t-test, and for (ii) with a

paired sample t-test. Based on the Sidak post-hoc correction for multiple testing, the formula $(1-(1-\alpha)^{1/nmt})$ was used with ‘α=0.05’ and ‘nmt’ being ‘number of multiple tests’. Then, the level of significance was defined (i) at $p<0.0253$, and (ii) at $p<0.0169$.

Results

Participants

Age, habitual behavior assessments and all other anthropometric characteristics were not different between HC and the subgroups of patients with T2DM. The overall patient group had a diabetes duration ranging from 2 to 31 years with a mean of 13 years and an average HbA1c value of 7.4% (±1.03) [26].

Furthermore, LBM^{arm} and LBM^{leg} did not differ between HC, dNP-, dNPs and dNPsm. Age, level of PA, DXA body composition data, use of medication and a list of T2DM related complications can be consulted in Supplementary table 1.

Table 1a displays the results of between-groups analyses of maximal IM muscle strength of the dominant hand (one-way ANOVA) and the relative maximal IM and IK muscle strength of the dominant elbow, knee and ankle (one-way analysis of covariance (ANCOVA) with LBM^{arm} and LBM^{leg} as respective covariates). Table 1b presents the absolute data of elbow, knee and ankle IK muscle endurance total work (one-way ANOVA). Post-hoc comparisons were performed by means of the Sidak test.

Table 1a: Maximal IM muscle strength of the dominant hand and relative maximal IM and IK muscle strength of the dominant elbow, knee and ankle

	HC (n=19)	**dNP-** (n=8)	**dNPs** (n=13)	**dNPsm** (n=14)
Maximal IM muscle strength				
HGS				
max *(kg)*	49.8 (±9.08)	42.5 (±5.71)	40.8 (±9.99)	**39.1 (±9.18)[a]**
Elbow extension				
max PT/LBM^{arm} *(Nm/kg)*	12.8 (±1.96)	10.6 (±1.25)	11.6 (±3.26)	10.7 (±2.54)
Elbow flexion				
max PT/LBM^{arm} *(Nm/kg)*	17.4 (±3.80)	14.4 (±3.16)	14.6 (±2.64)	**14.0 (±2.41)[a]**
Knee extension				
max PT/LBM^{leg} *(Nm/kg)*	15.5 (±3.05)	13.6 (±3.15)	12.9 (±2.94)	13.1 (±3.69)
Knee flexion				
max PT/LBM^{leg} *(Nm/kg)*	10.5 (±1.58)	8.5 (±1.40)	8.7 (±2.49)	8.9 (±2.25)
Ankle extension (PF)				
max PT/LBM^{leg} *(Nm/kg)*	9.2 (±2.70)	6.6 (±3.01)	7.5 (±1.64)	6.8 (±2.57)
Ankle flexion (DF)				
max PT/LBM^{leg} *(Nm/kg)*	3.3 (±1.17)	3.5 (±1.06)	2.7 (±1.08)	**2.0 (±0.71)[ab]**

Maximal IK muscle strength				
Elbow extension				
max PT/LBM^{arm} *(Nm/kg)*	12.6 (±2.87)	9.6 (±1.04)	10.5 (±3.63)	**8.8 (±1.82)[a]**
Elbow flexion				
max PT/LBM^{arm} *(Nm/kg)*	13.8 (±2.73)	12.0 (±2.16)	**11.3 (±2.14)[a]**	**11.0 (±1.75)[a]**
Knee extension				
max PT/LBM^{leg} *(Nm/kg)*	14.3 (±3.44)	11.9 (±2.86)	12.6 (±2.50)	11.7 (±3.26)
Knee flexion				
max PT/LBM^{leg} *(Nm/kg)*	7.4 (±1.36)	6.5 (±0.91)	6.3 (±1.21)	**5.8 (±1.73)[a]**
Ankle extension (PF)				
max PT/LBM^{leg} *(Nm/kg)*	5.8 (±2.04)	5.0 (±2.96)	3.5 (±1.24)	**2.6 (±1.45)[a]**
Ankle flexion (DF)				
max PT/LBM^{leg} *(Nm/kg)*	2.4 (±0.70)	2.2 (±0.50)	2.0 (±0.53)	**1.5 (±0.41)[a]**

All data are expressed as mean (±SD).

HC, healthy controls; dNP-, patients without diabetic NP; dNPs, patients with sensory dNP; dNPsm, patients with sensorimotor dNP.

IM, isometric; HGS, handgrip strength; PT, peak torque; LBM^{arm}, lean body mass of the dominant arm; LBM^{leg}, lean body mass of the dominant leg; PF, plantar flexion; DF dorsiflexion; IK, isokinetic.

a $p<0.05$ compared to HC

b $p<0.05$ compared to dNP-

Table 1b: IK muscle endurance of the dominant elbow, knee and ankle

	HC (n=19)	**dNP-** (n=8)	**dNPs** (n=13)	**dNPsm** (n=14)
Elbow extension				
total work *(J)*	841.1 (±236.06)	653.4 (±148.11)	**650.1 (±273.84)[a]**	**597.2 (±197.73)[a]**
Elbow flexion				
total work *(J)*	898.9 (±324.56)	698.4 (±116.98)	690.3 (±252.65)	**670.2 (±225.18)[a]**
Knee extension				
total work *(J)*	2124.8 (±480.33)	**1667.6 (±141.05)[a]**	**1761.3 (±480.04)[a]**	**1583.6 (±681.77)[a]**
Knee flexion				
total work *(J)*	998.9 (±291.43)	787.3 (±234.22)	**728.0 (±316.33)[a]**	**622.6 (±375.91)[a]**
Ankle extension (PF)				
total work *(J)*	252.6 (±80.27)	**103.9 (±55.32)[a]**	**100.4 (±60.12)[a]**	**70.3 (±92.84)[a]**
Ankle flexion (DF)				
total work *(J)*	82.0 (±26.02)	52.5 (±45.57)	47.0 (±44.81)	**24.1 (±29.74)[a]**

All data are expressed as mean (±SD).

HC, healthy controls; dNP-, patients without diabetic NP; dNPs, patients with sensory dNP; dNPsm, patients with sensorimotor dNP.

IK, isokinetic; PF, plantar flexion; DF dorsiflexion.

a $p<0.05$ compared to HC

In seven patients data for ankle PF and/or DF strength parameters were missing. As the data of all seven patients were similar to the baseline characteristics of the cohort, we decided to include their HGS, elbow and knee strength data in the final analysis (Supplementary table 2).

Distal versus proximal lower limb comparison between dNP-, dNPs, and dNPsm

At the ankle, total work ratios were more negative compared to the knee (p<0.001) with an effect size (Partial Eta Squared; η^2) of 0.629. In dNP-, dNPs and dNPsm, the paired sample t-test of the ankle-knee comparison revealed significant lower ankle values (resp. p=0.005, p=0.005, and p=0.001). As no significant joint*group interaction was detected, this effect was independent of the presence of dNP (p=0.555) (Table 2).

Significant lower maximal IK ankle ratios were found compared to the knee ratios (p=0.003; η^2=0.290). A significant joint*group interaction (p=0.049; η^2=0.200) for maximal IK ratios was observed, indicating that the most negative maximal IK ratios were dependent on the presence of dNPsm. Specifically, at the ankle, the dNPsm group was significantly more affected than the dNP- group (p=0.010) and, additionally, more negative values of the ankle compared to the knee were observed within the dNPsm groups (p<0.001) (Table 2).

For the IM muscle strength ratios, no significant differences were found in the ankle-knee comparison (Table 2).

Table 2: Ankle-knee comparison for IK muscle endurance total work, maximal IK and IM muscle strength

	dNP-	dNPs	dNPsm	ankle-knee comparison	joint*group interaction
total work ankle ratio *(%)*	**-53.3 (±18.93)[a]**	**-55.8 (±25.69)[a]**	**-71.8 (±33.94)[a]**	***p*<0.001**	*p*=0.555
total work knee ratio *(%)*	-21.4 (±10.90)	-20.3 (±21.97)	-29.4 (±32.71)		
max IK ankle ratio *(%)*	-12.5 (±38.97)	-32.7 (±16.94)	**-49.8 (±19.41)[a', b]**	***p*=0.003**	***p*=0.049**
max IK knee ratio *(%)*	-15.1 (±16.96)	-12.9 (±15.41)	-19.3 (±22.05)		
max IM ankle ratio *(%)*	-17.7 (±30.60)	-17.2 (±12.66)	-26.3 (±21.61)	*p*=0.258	*p*=0.240
max IM knee ratio *(%)*	-14.7 (±16.34)	-17.1 (±17.67)	-15.6 (±21.41)		

Data are expressed as mean (±SD).

IK, isokinetic; IM, isometric; dNP-, patients without diabetic NP; dNPs, patients with sensory dNP; dNPsm, patients with sensori-motor dNP.

a p<0.017 dNP-, dNPs, dNPsm: ankle compared to knee

a' dNPsm: ankle compared to knee

b p<0.025 ankle: dNPsm compared to dNP-

Distal versus proximal upper limb comparison between dNP-, dNPs, and dNPsm

The maximal IM HGS ratios *(%)* in the dNP-, dNPs and dNPsm group were respectively -14.6 (±11.47), -18.0 (±20.07) and -21.5 (±18.44). For maximal IM elbow strength in the dNP-, dNPs and dNPsm group, the ratios were respectively -17.4 (±13.25), -13.4 (±11.79) and -18.3 (±14.79). The distal versus proximal upper limb comparison for maximal IM muscle strength did not show any significant differences within (hand-elbow comparison; p=0.652) and between the different groups (joint*group interaction; p=0.725).

Upper versus lower limb comparison between dNP-, dNPs, and dNPsm (elbow-knee)

The IK muscle endurance elbow total work ratios *(%)* in the dNP-, dNPs and dNPsm group were respectively -22.3 (±14.58), -23.0 (±29.55) and -27.2 (±23.35). For IK muscle endurance knee total work in the dNP-, dNPs and dNPsm group, the ratios were respectively -21.4 (±10.90), -20.3 (±21.97) and -29.4 (±32.71). The upper versus lower limb comparison for IK muscle endurance total work did not show any significant differences within (elbow-knee comparison; p=0.922) and between the different groups (joint*group interaction; p=0.889).

The maximal IK elbow strength ratios *(%)* in the dNP-, dNPs and dNPsm group were respectively -18.1 (±11.17), -17.4 (±20.60) and -25.1 (±12.66). For maximal IK knee strength in the dNP-, dNPs and dNPsm group, the ratios were respectively -15.1 (±16.96), -12.9 (±15.41) and -19.3 (±22.05). The upper versus lower limb comparison for maximal IK muscle strength revealed no significant changes in any group (elbow-knee comparison; p=0.072), nor between groups (joint*group interaction; p=0.808).

The maximal IM elbow strength ratios *(%)* in the dNP-, dNPs and dNPsm group were respectively -17.4 (±13.25), -13.4 (±11.79) and -18.3 (±14.79). For maximal IM knee strength in the dNP-, dNPs and dNPsm group, the ratios were respectively -14.7 (±16.34), -17.1 (±17.67) and -15.6 (±21.41).The upper versus lower limb comparison for maximal IM muscle strength revealed no significant changes in any group (elbow-knee comparison; p=0.529), nor between groups (joint*group interaction; p=0.521).

Upper versus lower limb comparison between dNP-, dNPs, and dNPsm (hand-ankle)

The maximal IM HGS ratios *(%)* in the dNP-, dNPs and dNPsm group were respectively -14.6 (±11.47), -18.0 (±20.07) and -21.5 (±18.44). For maximal IM ankle strength in the dNP-, dNPs and dNPsm group, the ratios were respectively -17.7 (±30.60), -17.2 (±12.66) and -26.3 (±21.61). The distal versus proximal upper limb comparison for maximal IM muscle strength did not show any significant differences within (hand-ankle comparison; p=0.652) and between the different groups (joint*group interaction; p=0.725).

Discussion

The main objective of this study was to determine whether muscle weakness was more pronounced in the distal (ankle) compared to proximal (knee) part of the lower limb and in the distal (hand) compared to proximal (elbow) part of the upper limb in patients with T2DM and, if present, whether this was affected by the presence and severity of dNP.

For muscle endurance total work, the ankle ratios were significantly more negative compared to the knee ratios, independently of the presence of dNP. Concerning maximal IK strength, the ankle ratios were significantly more negative compared to the knee ratios, dependent on the presence of dNP as the lowest values were only present in the dNPsm group. Regarding the upper limb, no significant differences in ratios between subgroups were found. This might suggest a more pronounced impact of dNP on the distal compared to proximal muscles in the lower limb versus upper limb.

Distal versus proximal lower limb comparison between dNP-, dNPs, and dNPsm

The main finding in the lower limb is a more distinct muscle weakness in the ankle versus the knee. This is in line with the results of our previous research and certainly provides more in-depth information about the impact of dNP-, dNPs and dNPsm on lower limb muscle weakness [26].

This study revealed that muscle endurance of the ankle is more affected than the knee in all patients with T2DM, independent of the presence of dNP. The impact of chronic hyperglycemia and impaired glycemic control on top of the ageing process of skeletal muscle fibers should not be neglected. Furthermore, altered contractile mechanisms and cellular metabolism (e.g. insulin resistance, metabolic inflexibility, reduced mitochondrial function and accelerated advanced glycation end products) play a role in the deterioration of muscle endurance [39]. Allen et al., 2015, proposed that dNP-related loss of muscle endurance is partially attributed to neuromuscular transmission instability under conditions that stress the capacity of the system, such as fatiguing contractions, and to possibly pathological alterations in the above-mentioned cellular metabolism or blood flow [11,13]. Another approach to clarify the decreased muscle endurance in this population is the well-documented muscle fiber type shift in T2DM patients over the years towards a higher proportion of type II muscle fibers, knowing that the slow-twitch oxidative muscle fibers type I are predominantly activated by endurance stimuli [4, 5, 40-42]. Additionally, we hypothesize that smaller muscle groups at

smaller joints could be more vulnerable to metabolic changes than larger muscle groups at larger joints. The ankle and hand joints consist of smaller muscle groups with lower muscle mass, less adequate microvascular blood supply, and are possibly more affected due to mitochondrial dysfunction and impaired free fatty acid metabolism.

Interestingly, the maximal IK muscle strength of the ankle also revealed lower values compared to the knee, only in the dNPsm group. Hence, we postulate that, additional to the metabolic factors caused by the disease itself, the presence and severity of dNP has a negative impact on maximal muscle strength. This may be due to fiber length-dependent or progressive centripetal degeneration of peripheral nerve axons in combination with an impaired regeneration, causing length-dependent neurological complications. As sensory neurons are less resistant than motor neurons to the dysfunction and degeneration associated with the disease itself, injuries due to lack of sensation may be noticed before muscle strength decreases. Nevertheless, the damages to the sensory and motor nervous system progress in a distal-to-proximal way, generally starting at the toes, extending over the feet and sometimes spreading to/over the lower legs or higher above the knee level, depending on the intensity of the peripheral nerve lesions [10, 12-14, 18, 21, 43].

Finally, the reduced lower limb muscle strength in patients with T2DM may have a high impact on their functionality and mobility. Upper leg muscles are larger, bigger and stronger than lower leg muscles, and thereby play a more important role in gait and functional mobility [44]. However, the muscles of the ankle play a key role in the biomechanics of gait (e.g. foot to roll over from heel to toe in a natural way) and, consequently, have large impact on gait quality [14].

Distal versus proximal upper limb comparison between dNP-, dNPs, and dNPsm

No significant differences were found between the handgrip and the elbow strength, indicating that total upper limb muscles might not be as much influenced by dNP as lower limb muscles, apparently being more progressively affected. It might be assumed that upper limb muscles are better preserved than lower limb muscles, which may be due to the length-dependent differences in upper and lower nerves.

Upper versus lower limb comparison between dNP-, dNPs, and dNPsm

Purely based on the stronger reduction in muscle strength in ankle compared to knee and no significant differences between hand and elbow, it can be suggested that there is a different impact of dNP on upper and lower limb muscle strength. Unfortunately, this was not supported by the comparison of the maximal muscle strength of the reciprocal joints (elbow versus knee and hand versus ankle).

Often, when the loss of sensory axons and/or motor axons and units extends above the knee level, progressively, the fingers, hand and forearm can be affected too, following the same fiber length-dependent pattern as in the lower limbs. Occasionally, the neuropathy may even affect the sensory nerve fibers of the intercostal nerves [43].

Lynch et al., 1999, found a more distinct decline in the maximal peak torque of lower limb muscles compared to the peak torque of upper limb muscles, which was definitely age-related [45]. Ageing may induce more inherent morphological changes in the leg than in the arm and, therefore, leg muscles might be more susceptible to loss of lean muscle mass. Another possible explanation for the more intact upper limb in the elderly is the quantity (level and intensity) of the activities of daily living, performed by the arm muscles, such as dressing, cooking, bathing, rising from a chair or sitting down, and activities of self-care in general… [46-47].

Clinical implications

We investigated the maximal muscle strength and muscle endurance of lower and upper limbs, as these can be considered as important components of physical fitness and function. Minimum levels of both are needed to perform activities of daily living, to maintain functional independence while ageing, and to participate in active leisure-time activities without strains, stress or fatigue [48].

Generally, muscle weakness of lower limbs may definitely impact gait and balance, may increase risk of falls, and may negatively influence gait rehabilitation. Besides muscle weakness, the functional shortcoming in this ageing population can be caused by loss of proprioception, decreased joint mobility, and impaired vision [11, 12, 14]. Nowadays, the American Diabetes Association recommends aerobic exercises (e.g. walking and bicycling) in combination with gradually increased resistance exercises (e.g. exercises using machines and elastic resistance bands), predominantly in order to strengthen larger lower limb muscles to reduce risk factors such as insulin

resistance, cardiovascular components, and overweight [44, 49]. However, the majority of exercise therapy researchers lay focus on the musculature of the knee as they claim that knee extensors are a major antigravity muscle group, responsible for propelling and controlling the body during gait. Consequently, T2DM patients with dNP who experience knee muscle weakness can suffer from impaired balance, reduced gait speed, increased incidence of falls, and severe injuries with hospitalization [18, 44]. Besides the alterations in the cartilage, ligaments and tendons of the knee, an increased thickness of the Achilles tendon and plantar fascia has been observed, leading to decreased flexibility of the ankle joint and limited dorsiflexion during walking [18]. Therefore, future research should rather investigate the effect of a refined training program focusing on smaller muscle groups such as ankle and hand musculature. Optimized and strengthened muscles around the smaller joints are necessary for the patients' functionality in order to stay as mobile as possible. Initially, physical therapy should be concentrated on analytical exercises. Later on, this training program could be combined with a more functional approach to focus on the physical component.

Strengths and limitations

Dyck et al., 2010, showed that a clinical diagnosis of dNP is unreliable and inaccurate [50]. Therefore, we relied on the more accurate ENMG testing, which is and remains the gold standard for the diagnosis of dNP [3].

We decided to incorporate DXA data into our study, as this is described as the preferred method for both research and clinical use [34].

In our study, the Biodex® dynamometer was consequently used for all IM and IK elbow, knee and ankle assessments at the dominant side. However, as maximal IM HGS was executed by using the Jamar® dynamometer, it was difficult to compare HGS ratios with maximal IM elbow and/or ankle ratios. In future research, we recommend the use of the Biodex® dynamometer in order to assess maximal IM and IK wrist palmar flexion and dorsiflexion, and to compile muscle endurance total work results.

As already mentioned in our previous publication, the power of the results may be jeopardized by the limited number of dNP- patients compared to the dNPs and dNPsm groups [26].

In our study, the median age (min-max) in three of the four groups was approximately equal (HC 64 (55-76), dNP- 64 (61-70) and dNPs 66 (55-76)). Contrary to this, the

dNPsm group showed a wider range in age (67 (58-82)), albeit not significant different from the other groups. It is well known that healthy subjects reach their maximal muscle strength at the age of 30 with relatively stable values until the age of 60-65 [51, 52]. Thereafter, an age-dependent progressive loss of muscle mass and strength can be observed, described as 'sarcopenia', which can result in functional impairment leading to falls, injuries, and loss of independence in the healthy ageing population [34, 51, 52]. Meanwhile, in our target population, the diabetes health state should be taken into consideration as sarcopenia may occur earlier in patients with T2DM (often between 50 and 60 years), and as dNP on top of the age-dependent muscular degeneration could induce a synergistic detrimental impact on muscle mass and strength [51, 53].

As the Baecke questionnaire for activities of daily living was used in this study, we did not segregate the level and intensity of daily use of upper and lower limbs. In future research, the investigators could question the daily use of arms and legs separately to get more insight into differences in frequency and intensity.

Furthermore, the normalization method used in this study, may have biased the statistical analysis of the obtained results due to the absence of a large dataset in healthy, age-, weight-, and gender-matched controls [38].

Finally, muscle strength can also be influenced by nutritional status and musculoskeletal pain, which was not assessed in this study [26].

Future research directions

The design of future studies should rather be longitudinal and prospective as it is of very high importance to investigate the possibility of a distal-to-proximal progression of muscle weakness in lower and upper limbs of patients with T2DM, eventually due to the length-dependent nature of dNP, in order to preserve functionality and independence, and to reduce the risk of falls.

Conclusion

This study suggests a more pronounced weakness in the ankle compared to the knee regarding maximal muscle strength due to the presence and a gradient in severity of dNP. Moreover, this phenomenon was only present in the lower limb compared to the upper limb. Muscle endurance total work revealed significantly lower ankle ratios in all three diabetic groups compared to the knee, and thus independent of the presence of

dNP. Therefore, our research group suggests that metabolic disturbances in patients with T2DM are probably responsible for these negative values.
These findings are of major importance to construct optimal and appropriate analytical strength programs in lower and upper limbs in order to maintain functional independence, and in particular tailored to T2DM patients with or without dNP.

Disclosure

The data of this paper have been presented as a poster at the 56th Virtual EASD Annual Meeting, 21-25 September 2020.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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B.V.E., D.C. and P.C. conceived and designed the study, analyzed data, wrote, edited, and reviewed the manuscript. B.V.E., B.L., P.P., K.V.W., S.H. and J.S. researched data, contributed to the discussion and interpretation of data, and edited and reviewed the manuscript. All authors gave final approval for publication. B.V.E. and P.C. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Supplementary table 1: Additional information of the participants

	HC (n=19)	**dNP-** (n=8)	**dNPs** (n=13)	**dNPsm** (n=14)
Age *(years)*	64 (±6.7)	65 (±3.2)	66 (±6.9)	67 (±8.3)
Level of PA *(/15)*	8.0 (6.3-9.6)	8.5 (5.5-9.5)	8.0 (5.1-10.1)	7.6 (6.6-10.3)
LBM^{tot} *(kg)*	61.5 (±6.90)	66.1 (±9.57)	65.7 (±10.01)	68.7 (±8.41)
LBM^{arm} *(kg)*	3.6 (±0.56)	3.9 (±0.78)	3.8 (±0.76)	3.9 (±0.57)
LBM^{leg} *(kg)*	9.6 (±1.10)	10.0 (±1.50)	9.8 (±1.49)	10.1 (±1.50)
FM^{tot} *(kg)*	18.5 (±5.00)	22.3 (±5.80)	21.6 (±10.00)	23.4 (±6.47)
DM medication oral *(%)*	0	100	84.6	71.4
Metformin® *(%)*	0	62.5	69.2	42.9
Januvia® *(%)*	0	12.5	0	7.1
DM insulin injection *(%)*	0	37.5	50.0	85.7
Lantus® *(%)*	0	0	23.1	28.6
Humalog® *(%)*	0	12.5	0	7.1
Novorapid® *(%)*	0	12.5	15.4	14.3
Other medication *(%)*	57.9	87.5	69.2	78.6
NSAIDs *(%)*	0	12.5	0	0
Anticoagulants *(%)*	15.8	50.0	46.2	71.4
Cholesterol-lowering *(%)*	31.6	75.0	23.1	57.1
Antihypertensive *(%)*	26.3	62.5	53.8	71.4
DM complications other than dNP	0	2	2	5
retinopathic	0	0	0	1
nephropathic	0	0	1	2
cardiovascular	0	2	2	2
orthopedic (LJM)	0	1	0	0
dermatologic (ulcer)	0	0	0	2

Age, LBM and FM data are expressed as mean (±SD); level of PA is expressed as median (min-max).
The percentages of each participant's relevant medication intake are presented.
The number of patients with DM related complications (other than dNP) are presented.

HC, healthy controls without neuropathy; dNP-, patients without diabetic neuropathy (dNP); dNPs, patients with sensory dNP; dNPsm, patients with sensorimotor dNP.

PA, physical activity; LBM^{tot}, total lean body mass; LBM^{arm}, lean body mass of the dominant arm; LBM^{leg}, lean body mass of the dominant leg; FM^{tot}, total fat mass; DM, diabetes mellitus; NSAIDs, nonsteroidal anti-inflammatory drugs; LJM, limited joint mobility.

Supplementary table 2: Main characteristics of the seven participants with missing ankle DF/PF ratios

Patient	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**
ENMG	dNP-	dNPs	dNPs	dNPs	dNPsm	dNPsm	dNPsm
Age *(yrs)*	62	59	65	72	71	60	68
BMI *(kg/m^2)*	27.7	36.9	30.0	22.9	28.5	33.5	32.1
Diabetes duration *(yrs)*	3	13	10	10	6	26	10
HbA1c *(%)*	6.0	7.1	6.6	7.2	5.5	10.0	8.0
LBM^{arm} *(kg)*	4.0	5.2	3.8	3.7	3.9	3.9	3.3
LBM^{leg} *(kg)*	11.0	12.4	9.3	8.6	9.1	11.0	9.8

DF, dorsiflexion; PF, plantar flexion; ENMG, electroneuromyography; dNP-, patients without diabetic NP; dNPs, patients with sensory dNP; dNPsm, patients with sensorimotor dNP; BMI, body mass index; LBM^{tot}, total lean body mass; LBM^{arm}, lean body mass of the dominant arm; LBM^{leg}, lean body mass of the dominant leg.

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PART III

DISCUSSION AND FUTURE PERSPECTIVES

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1. Main findings

This dissertation aimed on the exploration of the impact of dNP on respiratory and peripheral muscle strength in patients with T2DM.

First aim: The influence of clinically diagnosed neuropathy on respiratory muscle strength in type 2 diabetes mellitus. *PI_{max}, PE_{max}* and *PEF* were significantly lower in D+ compared to C; in addition, PE_{max} and PEF were also significantly lower in D- versus C. Comparing both respiratory muscle strength parameters, PI_{max} as well as PE_{max} were predominantly impacted by the presence of dNP (respectively -43% and -35%), however this impact was less pronounced in PE_{max}, probably due to the minor muscular effort during the breathing-out maneuver.

Second aim: The impact of sensory and/or sensorimotor neuropathy on lower limb muscle endurance, explosive and maximal muscle strength in patients with type 2 diabetes mellitus. *Maximal muscle strength* in the lower limbs of patients with T2DM was definitely influenced by the presence and the severity of dNP. The maximal IK peak torque of knee flexion, ankle PF and DF was significantly lower in the dNPsm group, compared to HC. *Explosive muscle strength* was negatively impacted by the presence and severity of dNP, and, as such to a certain extent comparable with the impact on maximal muscle strength outcomes. Regarding *muscle endurance* in ankle and knee, a different pattern was identified compared to the other strength parameters. Lower values were observed in all T2DM patients (without or with dNP) compared to HC, indicating that the disease itself rather than dNP affects this strength parameter.

Third aim: The impact of diabetic neuropathy on the distal versus proximal comparison of weakness in lower and upper limb muscles of patients with type 2 diabetes mellitus: a cross-sectional study. For muscle endurance total work, the ankle ratios were significantly more negative compared to the knee ratios, independently of the presence of dNP. Concerning maximal IK strength, the ankle ratios were significantly more negative compared to the knee ratios, dependent on the presence of dNP as the lowest values were only present in the dNPsm group. Regarding the upper limb, no significant differences in ratios between subgroups were found. This might suggest a more pronounced impact of dNP on *the distal compared to proximal muscles* in the *lower limb* versus *upper limb*.

2. Critical reflection on main findings

2.1. The impact of diabetic neuropathy on maximal respiratory muscle strength

T2DM as such seems to be a risk factor for accelerated decline in respiratory muscle strength and pulmonary function. Mechanisms likely explaining this phenomenon are the negative effects of hyperglycemia on alveolar barrier conductance, the potential *impact of dNP* and/or respiratory muscle dysfunction on lung expansion, and a greater incidence and severity of respiratory infections [1]. Fuso et al., 2012, included patients with non-specified dNP (12%), nephropathy (13%) and retinopathy (16%), and found a significant reduction of PI_{max} in all patients with T2DM compared to HC, whereas no differences were observed in PE_{max} [1]. Another study by Kaminski et al., 2011, specifically compared patients with autonomic cardiovascular dNP with HC, and also revealed lower PI_{max} (-22%) in the particular patient group compared to HC with similar PE_{max} values in both groups [2]. These results are broadly in line with our key findings, revealing lower PI_{max} and PE_{max} measures in the patient groups without or with clinically diagnosed dNP compared to HC. Consequently, every single research provides evidence of a negative effect of T2DM as such on the maximal respiratory muscle strength.

In order to understand the impact of T2DM, muscle fiber type distribution and vascularization of the respiratory muscles have to be explored in depth. Checking on the muscle fiber type distribution of the diaphragm in healthy humans, the mean relative occurrence of type I fibers is approximately 50%. The remaining proportion is equally divided into type IIa and IIx fibers. Both the inspiratory and expiratory intercostal muscles have at least 10% more type I fibers than the diaphragm and most other skeletal muscles, whereas the expiratory internal intercostal muscles show an almost complete lack of type IIx fibers [3]. Furthermore, all muscle fibers are surrounded by a certain number of capillaries, depending on their fiber type. In the diaphragm, type I fibers are surrounded by 4-6 capillaries per fiber, whereas slightly less (3-5) are found around type IIa and IIx fibers. However, the calculated values for the fiber area surrounded by each capillary are smaller in the diaphragm than in leg or arm muscles. In the expiratory intercostal muscles, more capillaries are found around both type I and type IIa fibers (5-6) compared to the inspiratory intercostal muscles (4-5) [3]. However, the impact of T2DM on the maximal respiratory muscle strength needs further and

more profound investigation as little consideration has been taken into account for the preservation of respiratory muscle strength and the prevention of respiratory muscle weakness.

2.2. The impact of diabetic neuropathy on maximal and explosive muscle strength in lower and upper limb

A large body of evidence has shown that T2DM is associated with multiple deteriorating muscular changes, such as reduced maximal muscle strength, explosive muscle strength/power, muscle endurance, muscle mass and muscle quality (i.e. maximal voluntary contractile strength or torque per unit regional muscle mass of the specific body compartment [4]), and an altered fiber type composition which affects the upper and especially the lower limbs. The concept that hyperglycemia directly impacts on the intrinsic properties of the muscles to generate force is a growing field of interest. Hence, T2DM seems to be responsible for the decrease in (neuro)muscular performance, and, consequently, for overall skeletal muscle strength deterioration. Additionally, chronic complications of T2DM, particularly dNP, have been ascertained to implicate the pathogenesis of neuromuscular impairment. Thus, this neuromuscular dysfunction is initially caused by long-term exposure to hyperglycemia, but is definitely further aggravated by the presence and severity of neuropathic complications due to reduced action potentials and impaired nerve conduction velocity [5, 6]. The more absolute force has to be generated (e.g. maximal muscle strength) and the faster the movements have to take place (e.g. explosive strength), the bigger the distressing impact of dNP on the given strength parameters.

Regarding the interference of previous changes to hyperglycemia by dNP, we strongly believe that *maximal and explosive muscle strength* in patients with dNP are susceptible to additional metabolic and neurogenic alterations, which eventually may lead to a further loss of peripheral skeletal muscle strength. The irreversible nerve damage causes changes in the neuromuscular system and motor function, and contributes to the decrease of maximal muscle strength and muscle atrophy in the lower limbs [5]. The early selective injury of sensory and motor axons in dNP may be unraveled by three following steps. First, all axons seem to be dependent on Schwann cells as a source of energy, which during the development of T2DM not only lose their ability to provide energy to myelinated and unmyelinated axons, but also transfer toxic lipid species to the axons they contact, making the axons particularly vulnerable.

At this point, the role of the mitochondria has to be delineated and it has to be recognized that mitochondrial integrity, motility, and localization along the axon are all adversely affected by T2DM. For instance, the Schwann cells produce acetyl-carnitines who trigger the influx of extracellular Ca^{2+} into the axon that impairs axonal mitochondrial trafficking, resulting in insufficient axonal energy production and mitochondrial apoptosis in T2DM. Second, axons are highly vulnerable to T2DM-mediated injury due to their abundant expression of ion channels with a number of distinct voltage gated sodium channels as well as the Na^{+}-Ca^{2+} exchanger isoform 2 in their terminals. When the Na^{+}-K^{+} ATPase levels, required to export intra-axonal Na^{+} in order to accumulate following action potential propagation, are below normal, this leads in turn to increased intra-axonal Na^{+}, increased intracellular Ca^{2+} and axonal degeneration. These two findings emphasize the vulnerability of axons to energetic stress as seen in the T2DM pathogenesis. Last, as sensory axons express distinct voltage gated sodium channels compared to motor actions and as these channels in sensory and motor axons have different biophysical characteristics, the sensory axons are more vulnerable to T2DM-mediated injury. Additionally, the sensory neurons have substantially smaller diameter axons compared to motor axons, resulting in a higher surface area to volume ratio, that can in turn accentuate changes in intracellular Na^{+} and Ca^{2+}, rendering sensory axons more susceptible to injury. Differences in conduction velocity properties and energy requirements also predispose unmyelinated or thinly myelinated sensory neurons to be more vulnerable to higher energy demands, that cannot be met in the presence of T2DM [7].

2.3. The impact of diabetic neuropathy on muscle endurance in lower and upper limb

As *muscle endurance* deterioration is broadly observed in all patients with T2DM, independently of the presence of dNP, it is more likely that this impairment is elicited by the T2DM pathogenesis as such. It is suggested that defective muscle endurance is another typical component of diabetic neuromuscular dysfunction in patients without or with dNP [5, 8, 9]. Unfortunately, the question of whether or not this is dependent on, or aggravated by, motor nerve damage has not been directly answered. Additionally, T2DM may affect both appendicular muscle mass and fiber composition in skeletal muscles [6]. As yet, no mechanisms were found that could possibly fully explain reduced muscle endurance in patients with T2DM. Independently of diabetic complications,

T2DM has been shown to be responsible for an impaired bio-energetical capacity of the skeletal muscle mitochondria and dysfunction of the sarcoplasmic reticulum [10]. The alterations in the mitochondrial morphology (smaller), density and function (increased production of mitochondrial ROS) cause a reduction in the oxidative enzyme capacity [10-12]. Both structural and functional changes in the mitochondria lead to oxidative stress, causing modifications in their genetic material. The repeated oxidative damage to the mitochondrial genetic material is responsible for mitochondrial dysfunction. This, again and in turn, causes an increase in ROS, which results in a vicious cycle of oxidative damage within the mitochondrion and, consequently, which can eventually lead to the induction of apoptosis and cell death [11, 12]. The dysfunction of the sarcoplasmic reticulum is the result of an impairment of Ca^{2+} handling of the sarcoplasmic reticulum itself and is caused by a degeneration of sarcoplasmic proteins due to the insulin resistance in patients with T2DM, leading to loss of muscle strength and endurance [5, 13]. These changes are also correlated with the disease duration and the quality of serum glucose control, as indicated by HbA1c levels [8].

The lower outcome in muscle endurance in patients with T2DM compared to healthy controls is possibly related to changes in both muscle fiber specific metabolism and muscle fiber composition in the diabetes population [10], manifesting in a decrease of slow oxidative type I muscle fibers and increased fast glycolytic type IIx fibers. This shift is likely to be responsible for a reduced oxidative capacity and an increased glycolytic capacity in the skeletal muscles of these patients [14]. Hence, this transformation in muscle fiber type distribution could also explain the reduced muscle endurance, as type IIx muscle fibers are less fatigue-resistant compared to type I muscle fibers due to a lower amount of mitochondria and capillaries [9].

Three possible explanations can be found why the muscle endurance data between the diabetes groups show no significant differences. First, in our studies, lean body mass (LBM^{tot}, LBM^{arm} and LBM^{leg}) between dNP-, dNPs and dNPsm was not significantly different, which may be the reason why all patients with T2DM show reduced muscle endurance in approximately the same ratio. Second, it is commonly known that muscle endurance assessment is conducted in a submaximal way, which may lead to submaximal activation of the motor units in the investigated muscles [15]. Last, in 2020, Orlando et al. investigated the relation between diabetic long-term complications (vascular and nerve dysfunctions) and muscle endurance of the knee extensors in patients with T2DM, revealing an independent association with dNP,

cardiovascular diseases and retinopathy. It has been suggested that dNP-related loss of muscle endurance is partially attributed to neuromuscular transmission failure (slowing contractile properties, reduced motor unit firing rates, and motor unit loss) and possible pathological alterations in muscle metabolism or blood flow [8, 16].

2.4. The impact of diabetic neuropathy on the distal versus proximal comparison of weakness in lower and upper limb muscles

The neuropathic complications in patients with T2DM play a determinant role in the denervation of muscle fibers in combination with insufficient re-innervation, leading to progressive and continuous loss of motor axons, an alteration of motor unit properties, alterations in the neuromuscular junction and in its transition, slowed muscle contractile properties, reduced motor unit firing rates, excess fat infiltration and structural changes in muscle fibers. These detrimental effects may result in loss of muscle mass and function, which is intrinsically associated with muscular atrophy and reduced muscle strength, power and quality of the upper and especially the lower limbs [6, 17-20].

Some researchers observed a negative effect of dNP (an accelerated decline) on the appendicular muscle mass and strength at an earlier stage in the feet and slowly but invariably progressing to the lower legs, presuming a *more pronounced impact of dNP on the distal compared to proximal muscles* in patients with T2DM [5, 6, 20]. For greater certainty, this decline is related to the degree of dNP and is clearly more pronounced distally, which supports the concept that the neuropathic process might depend on the length of the nerve [5]. In one of our studies, a similar impact of dNP on the distal compared to proximal muscles was detected for maximal muscle strength as well as for muscle endurance in the lower limbs, with worse strength values at the ankles compared to the knees (see Part II, pp 85-108). As known, the triceps surae and tibialis anterior, two relatively “large” muscles in the ankle region, are mainly involved in the PF and DF of the ankle. However, seven relatively small muscles whose tendons cross the joint (flexor hallucis longus, flexor digitorum longus, tibialis posterior, extensor hallucis longus, extensor digitorum longus, peroneus longus and brevis) endorse these movements, besides the e- and inversion of the ankle [21]. Compared to the ankle, knee extension is achieved by the quadriceps femoris muscle group (rectus femoris and vastus lateralis, medius and intermedius), while the hamstring group (semitendinosus, semimembranosus, and biceps femoris) flex the knee and extend the hip.

Both groups are much larger, powerful and stronger compared to the muscles around the ankle [22]. Furthermore, the fiber type within each muscle and the assumed muscle fiber type shift in patients with T2DM may also have impact on this process. It is well known that skeletal muscle fiber type composition is heterogeneous ranging from muscles consisting out of predominantly type I fibers (M. Soleus), over circa equally distributed type I and type II fibers (M. Gastrocnemius, knee extensors and flexors) to mainly type II fibers (M. Tibialis anterior) [5, 14, 23-26]. Yet it is unknown if the upper or the lower leg musculature in diabetics, with an unambiguous muscle fiber switch to type II and consequently an increase in glycolytic muscle capacity or anaerobic activity, is more susceptible to significant changes in muscle endurance capacity due to a distinct difference in recruitment ability of muscle fiber contraction considering the variation in the amount of fibers within each muscle [27].

Contrary to the findings in the lower limb, the impact of dNP on the distal compared to proximal muscles in the upper limb was definitely less pronounced, which can be explained by earlier research. A few articles described the development of dNP from the feet/ankles towards the knees due to the length-dependent nature of this neurological complication, whereas the hand and elbow strength was preserved for a longer time. Some researchers even claim that only when dNP progresses to the level of the knees, the upper limbs might be affected too [17, 28-30]. This delayed process of dNP in upper compared to lower limbs may possibly be explained by differences in nerve length, affecting the longest nerves first (legs) before affecting shorter ones (arms), and before progressing proximally in both limbs [31]. Additionally to this, we hypothesize that smaller muscle groups at smaller joints could be more vulnerable for metabolic changes than larger muscle groups at larger joints. As a logical consequence of this hypothesis, the ankle joint and the hand do consist of smaller muscle groups with lower muscle mass, higher quantity of microcirculation -more vulnerable to hyperglycemia-, and are possibly more affected due to mitochondrial dysfunction and impaired free fatty acid metabolism. The age-associated reduction in physical activity may be at least partially responsible for the change in muscle fiber type distribution with age. It is reasonable to assume that a reduction in physical activity would primarily be associated with a decreased use of lower body muscles, but not upper body muscles, given that the muscles in the lower body are required for most common activities (e.g. walking, stair climbing, …) [32].

2.5. Discrepancy between lower and upper limb strength in diabetic neuropathy

Besides the observed distal-to-proximal pattern of dNP in the lower limb, *a discrepancy in maximal muscle strength between lower and upper limb* was found. Actually, diabetic neuromuscular dysfunction affects maximal muscle strength in the leg more than in the arm due to differences in insulin sensitivity and mitochondrial (dys)function among several muscular areas, and the selective effect of dNP on the peripheral motor nerve function as possible causes for this regional effect [6]. Additionally, *a discrepancy in muscle endurance between lower and upper limb* was found. Orlando et al., 2017, assessed muscle endurance of the shoulder and knee extensors in patients with uncomplicated T2DM (i.e. absence of dNP, cardiovascular disease, retino- and nephropathy) and concluded that reduced muscle endurance is an important component of muscular dysfunction in T2DM and that it not only affects the lower limb, as observed for maximal muscle strength, but also the upper limb, suggesting that muscle fatigability may be more sensitive to the damaging effect of T2DM than maximal muscle strength. It has to be taken into account that these findings were based on an isometric evaluation (endurance time at 50% of the maximal voluntary contraction) and that the nature of the motor task, meaning the type of contraction (IM or IK), can influence the outcome of the tests [10].

2.6. Post-factum analysis on the data gathered throughout the research

Table 2 provides an overview of all gathered data in this dissertation. Compared to the healthy control group, an important decline in PI_{max} could be noticed in diabetic patients with dNP and in PE_{max} and PEF in all patients with T2DM, whereas a decrease in maximal and explosive muscle strength in the lower and upper limbs could only be identified in one subgroup of patients with dNP, i.e. dNPsm. In contrast, the muscle endurance data were significantly lower in all diabetic groups (without or with dNP). Please note that, in the respiratory study, no distinction was made between dNPs and dNPsm, which could average out the impact of sensory (tending towards D-) and sensorimotor dNP (with stronger impact) on the respiratory data.

Table 2 Post-factum analysis on the respiratory and peripheral muscle data

RESPIRATORY	**C-**	**D-**		**D+**
PI_{max}				***-43%****
PE_{max}		-31%*		**-35%***
PEF		-30%*		**-33%***
PERIPHERAL				
Maximal IM	**HC**	**dNP-**	**dNPs**	**dNPsm**
knee ext/flex				-15%
ankle PF/DF				***-30%****
elbow ext/flex				**-18%***
HGS				**-21%***
Maximal IK	**HC**	**dNP-**	**dNPs**	**dNPsm**
knee ext/flex				**-19%***
ankle PF/DF				***-50%****
elbow ext/flex				**-25%***
Explosive strength	**HC**	**dNP-**	**dNPs**	**dNPsm**
s2LJT				**-30%***
CRT				-20%
Endurance IK	**HC**	**dNP-**	**dNPs**	**dNPsm**
knee ext/flex		**-21%***	**-20%***	**-29%***
ankle PF/DF		***-53%****	***-56%****	***-72%****
elbow ext/flex		-22%	**-23%***	**-27%***

C-/HC, healthy controls without neuropathy; D-/dNP-, T2DM patients without diabetic neuropathy (dNP), D+, patients with dNP; dNPs, patients with sensory dNP; dNPsm, patients with sensorimotor dNP; PI_{max}, maximal inspiratory pressure; PE_{max}, maximal expiratory pressure; PEF, peak expiratory flow; IM, isometric; ext, extension; flex, flexion; PF, plantar flexion; DF, dorsiflexion; HGS, handgrip strength; IK, isokinetic; s2LJT, single two leg jump test; CRT, chair rising test.

* $p<0.05$ compared to C/HC

The most striking findings of this dissertation are (1) the significant declines in *PI_{max}, PE_{max} and PEF* compared to the healthy control group, (2) the neuropathy-induced decrease of *maximal and explosive muscle strength*, and reduced *muscle endurance* due to T2DM in the lower limbs, with an observed distal-to-proximal deterioration in the ankle versus knee, albeit only on a cross-sectional base and not by means of longitudinal research.

(1) A meta-analysis by van den Borst et al., 2010, provided indication that, similar to the cardiovascular system, the lung is a target organ in the systemic inflammatory process among patients with T2DM [33]. Respiratory dysfunction and muscle weakness in patients with T2DM can be detected and deduced in the general practitioner's office by analyzing the PEF value (based on spirometry or measured with a portable PEF

meter), as this parameter is determined by the strength (or the weakness) of the respiratory muscles [13]. When deviant values (more than -1SD) are recorded, the patient could be referred to a pulmonary specialist to eliminate or confirm some chronic underlying respiratory illness, or this deteriorated values could be an indication of latent dNP. In this case, it should be preferable to further investigate the presence and severity of dNP by ENMG (dNPs or dNPsm) or by corneal confocal microscopy (early detection). In our research, the gold standard in the detection of sensory or sensorimotor dNP was ENMG, as large myelinated lower and upper limb nerves were assessed profoundly in order to allocate the patients with T2DM into three different categories. However, this technique does not reveal the real onset of dNP, starting with dysfunction of small neuron fibers in the eyes, amongst others. Therefore, corneal confocal microscopy is a new standard technique with the ability to detect the first signs of dNP in a more precise way, which may result in an improved identification of patients without any sign of dNP (dNP-) and those with early onset dNP (dNPs).

(2) Looking at the results of our research, the muscles who are responsible for maximal ankle PF and DF and have great impact on gait, walking speed, quality of life, risk of falls, fractures, hospitalization, morbidity and mortality, … are mostly affected by dNP. In addition, it is of utmost importance that all patients with T2DM should focus on the preservation of the ankle mobility and on muscle endurance of the ankle joint. Functional tests, like the timed up and go, chair rising tests, SPPB, jump tests, … with special focus on the ankle can be easily implemented in clinical practice in an attempt to early screen and detect dNP.

Consequently, this dissertation highlights the need for physical therapy in all patients with T2DM, and an individualized treatment approach based on the presence and severity of dNP in the lower (focus on the ankle) and upper (focus on the hand) limbs. In particular, these two joints are of utmost importance to maintain overall functionality, e.g. gait, climbing stairs while holding the banister, toothbrushing, …

Further investigation is necessary to develop an extended and optimized multidisciplinary approach between general practitioners, physical therapists, specialists (endocrinologists, pulmonologists, ophthalmologists, physical specialists, neurologists, …) and diabetes educators in order to optimize the rehabilitation and to enhance the quality of life of all patients with T2DM and, in particular, those with sensorimotor dNP.

Each patient with mild, moderate or severe dNP could benefit from respiratory muscle strengthening exercises and from a patient-tailored strengthening program for the lower and upper limbs (see Chapter 'Clinical implications').

2.7. Strengths and limitations

In the first of the three original articles the diagnosis of dNP was solely based on clinical neurological examination, labeled 'clinically diagnosed NP (cdNP)'. This non-invasive and painless screening tool enabled the researchers to recruit a large number of participants (N=190), divided into T2DM patients without dNP (D-; n=28), patients with clinically diagnosed NP (D+; n=82), healthy controls without NP (C-; n=35), and controls with NP (C+; n=45) (see Part II, pp 42-62).

However, as clinically diagnosed dNP can be variable and rather inaccurate [34], in the next two articles we decided to rely on the more accurate ENMG testing, which is still considered as the gold standard for the diagnosis of dNP [35]. Consequently, we could make a substratification from (HC, dNP- and) dNP+ into (HC, dNP-,) dNPs and dNPsm with the opportunity to create in-depth analyses. A minor drawback here was the inability to divide the patients equally into the three diabetic groups, as it was not possible to forecast up front the presence and severity of dNP. In the 'Future research perspectives' chapter, new insight in the classification of the diabetic population into five replicable clusters has been presented, which may allow future research to divide this patient group more adequately and more unambiguously. Furthermore, the above mentioned chapter also reveals new insight into the innovating technique 'corneal confocal microscopy' as to indicate early onset of neuropathy, which may possibly replace the previously recognized 'gold standard' ENMG in the future.

Respiratory assessment by means of a Pocket-Spiro Mouth Pressure Monitor is feasible in the daily practice of generalists and specialists. Both PI_{max} and PE_{max} can be considered as an added value in a clinical non-invasive screening for the presence of dNP. Furthermore, similar profiles between respiratory and peripheral muscle function could be determined.

Nevertheless, we have to take into account that ±40% of the PI_{max} data in the healthy control group (15 men versus 20 women, and 25 community-dwelling older people versus 10 persons in a residential care setting) did not meet the age-related reference values, regretfully without an explanatory reason.

Additionally, in the first original research, we decided not to take the C+ group into consideration as non-diabetic NP did not belong within our field of interest. Consequently, this study focused only on three groups: D-, D+ and healthy controls (C); so all assessments performed on the C+ group (n=45) and all gathered data were withheld.

In the second and third original research, a minor drawback was the inability to divide the patients equally into the dNP-, dNPs, an dNPsm groups, as it was not possible to forecast up front the presence and severity of dNP.

The standard procedure for the measurement of skeletal lower and upper limb maximal muscle strength and muscle endurance consists of IM and/or IK dynamometry. Hence, the decision was made to use the Biodex® dynamometer for the assessment of ankle, knee and elbow strength. Only the measurement of the HGS was performed by means of the Jamar® dynamometer. In hindsight, no conscientious use of the same methodology in all joints (ankle, knee, elbow and hand) took place, which led to minor quality of the comparisons between ankle and hand, and between elbow and hand. Future research should probably benefit from the Biodex® dynamometry of wrist palmar flexion and dorsiflexion instead of the Jamar® dynamometry of HGS. In addition, muscle endurance of the HGS, also obtained by the Jamar® dynamometer, was expressed in seconds (the time elapsed from the maximal HGS until half its maximal value, a highly reproducible test in elderly subjects) which was obviously not compatible with the muscle endurance results of the Biodex® assessments.

Furthermore, it has to be mentioned that the impact of dNP on functionality and mobility has not been consistently investigated. Additionally, muscle strength can also be influenced by a variety of factors, including global nutritional status (dietary protein supplementation), possible vitamin D deficiency, hypogonadism, neuromuscular pain, and/or the use of some medication (e.g. statins, potentially causing musculoskeletal pain, and antidiabetic drugs per se) [36-42]. However, these variables were not included as confounding factors in our analyses, which may limit the interpretation of our results. Particularly the reporting on oral antidiabetic drug intake and/or injectable agents could have been described more profoundly in order to reflect on the impact of these drugs on the muscle parameters. It has been demonstrated that the drugs themselves have impact on sarcopenia, muscle mass and muscle strength in patients with T2DM [43-45].

Table 3 provides an overview of the positive and negative effects of all different antidiabetic agents [43, 46, 47].

Moreover, previous studies on SGLT2-i intake and GLP-1RA injections reported 25%-33% loss of body weight due to muscle loss [44], whereas oral metformin intake and insulin injections, the two most reported antidiabetic agents used by the diabetic population in original research 2 and 3 (see Part II, p 80 - Appendix Table A.2: Medication; p 104 – Supplementary table 1: Additional information of the participants), did not seem to negatively influence muscle mass and muscle strength. However, in view of the lifelong use of antidiabetic drugs in most patients, it is important to mention that metformin as well as insulin therapy could cause some negative effects. Metformin could attenuate anabolic effects, could cause protein degradation and autophagic cell death probably resulting in atrophy, whereas insulin therapy could fail to prevent skeletal muscle atrophy [46, 47]. Consequently, further research is needed to determine whether T2DM itself or the prescribed medication is the greater contributor to sarcopenia. In fact, the choice of antidiabetic drugs should take the risk of sarcopenia into consideration, in addition to comprehensive consideration of glycemic status and cardiovascular complications [43, 46].

Table 3 Overview on the effects of the different antidiabetic oral and injectable agents

ORAL	positive effects	negative effects
Metformin (first line)	peripheral insulin sensitivity ↑ insulin action in skeletal muscles ↑ muscle lipid accumulation ↓ mitochondrial biogenesis ↑ angiogenesis ↑ oxidative muscle fibers preservation lower limb strength ↑ potential LBM loss ↓ muscle mass and strength ↑	anabolic effects ↓ may potentiate the physiologically regulated autophagic-atrophy protein degradation
Sulfonylurea	insulin release in the β-cells (closure of the K_{ATP} channels) microvascular complications ↓	myopreservation ↓ vasodilatation ↓ cytoprotection ↓ islet cell protection ↓ ⇒ β-cell apoptosis ⇒ β-cell mass ↓ hypoglycemia moderate BW ↑ drug-induced atrophy

Glitazones (TZD)	IR in liver and peripheral tissues ↓ glucose production in liver ↓ muscle protein degradation ↓ intramyocellular lipid content ↓ fatty acid metabolism ↑ ⇒ energy metabolism ↑	hypoglycemia BW ↑ unknown effects on sarcopenia-related parameters
SGLT2-i	glucose reabsorption from the kidneys ↓ glucose excretion of in urine ↑ cardio- and renoprotective effects ↑	hypoglycemia urogenital infections dehydration ketoacidosis may induce or even accelerate diabetic-induced sarcopenia potential LBM ↓ muscle mass ↓ muscle strength ↓
DPP4-i	inactivation of the enzyme DPP-4 stimulation of incretin hormone GLP-1 ⇒ gastric emptying ↓ ⇒ glucagon release from α-cells ↓ ⇒ secretion of insulin in β-cells ↑	hypoglycemia in association with sulfonylurea or insulin
INJECTIONS	**positive effects**	**negative effects**
GLP-1RA	incretin-mimeticum analogue of incretin hormone GLP-1 ⇒ gastric emptying ↓ ⇒ glucagon release from α-cells ↓ ⇒ secretion of insulin in β-cells ↑ protective effect on muscle function	hypoglycemia in association with sulfonylurea or insulin BW ↓ FFM ↓
Insulin	human insulin or insulin-analogues muscle mass and strength ↑ attenuation of sarcopenia deterioration potent stimulatory factor for muscle protein synthesis	hypoglycemia infrequently BW ↑↑↑ prevention of atrophy ↓

LBM, lean body mass; K_{ATP} channel, ATP-sensitive potassium channel; BW, body weight; TZD, Thiazolidinediones; SGLT2-i, sodium-glucose cotransporter 2 inhibitors; DPP4-i, dipeptidylpeptidase-4 inhibitors; GLP-1RA, glucagon-like peptide-1 receptor agonist; FFM, fat free mass.

3. Clinical implications

To initiate this paragraph, some words of explanation must be given considering ageing in general. Lifetime peak muscle mass is suggested to occur between the age of 20 and 30 years and the rate of age-associated decline in muscle mass/strength is partly genetically determined [48]. However, the age-related decline in muscle mass, which is a major determinant of muscle strength, starts approximately at the age of 40 years, and in the years between the range of '40-44' and '75-79', men may lose 10.8% of muscle mass and women 6.4%. This ongoing degenerative loss of muscle mass and strength associated with ageing is called sarcopenia and has been reported to be an independent risk factor contributing to the onset or exacerbation of T2DM. Moreover, patients with T2DM have lower muscle mass and strength compared to non-diabetic elderly of the same age, indicating an acceleration of sarcopenia. Additionally, gradual loss of muscle strength in patients with T2DM is related to the presence and increased severity of dNP. So, on top of age-related sarcopenia and T2DM, the presence of dNP is a significant risk factor for reduced skeletal muscle strength, which might result in a decline in functionality and ADL [49-51].

Hence, taking the above mentioned cascade of age-related muscle mass and strength decline into consideration, it is abundantly clear that the treatment of sarcopenia itself can play an important role in the overall treatment of elderly patients with T2DM.

This dissertation highlights the need for an individualized treatment approach in T2DM. Both exercise training and physical activity have been demonstrated to be important non-pharmacological tools for the management of T2DM and have been considered a cornerstone in the prevention and treatment of T2DM due to many benefits, such as decreasing insulin resistance, and improving glycemic control, cardiorespiratory function, muscle strength, body composition and fat metabolism [51-53]. Consequently, both modalities effectively prevent (or at least delay) the onset of complications in patients with T2DM, and maintain motor function and skeletal muscle strength. Without compromising the importance of the effects of physical activity on physical function, the scope of this dissertation definitely lies on exercise training.

Among all exercise training modalities, *aerobic exercise* is traditionally the most-studied exercise program as it meets all above mentioned benefits by recruiting large muscle groups, with a patient-tailored intensity and exercise load under the guidance and monitoring of exercise experts. *Resistance training* is the preferred method to

prevent and manage sarcopenia by functionally or analytically applying muscle strength activity, whether or not isolated and brief. This method has been reported as a potential effective means of improving muscle strength and physical function. Yet, even better results can be expected by *combining aerobic exercise with resistance training* with positive synergistic effects on blood glucose control and insulin sensitivity, which is nowadays well established as a fundamental treatment in patients with T2DM [51, 54-57]. Actually, the aerobic component of the combined training may stimulate the resistance training because of its positive neuromuscular effect (e.g. muscle strength, sensorimotor function and dNP symptoms) and metabolic adaptations (e.g. reduction of cardiovascular risk, increased cardiorespiratory function, and aerobic and oxidative capacity) (see Figure 4) [6]. In its turn, a well-designed, progressive resistance exercise training program exerts positive effects on both nervous and muscular systems and, ultimately, results in profound enhancements in skeletal muscle mass and muscle strength, power and endurance (see Figure 4) [6, 58].

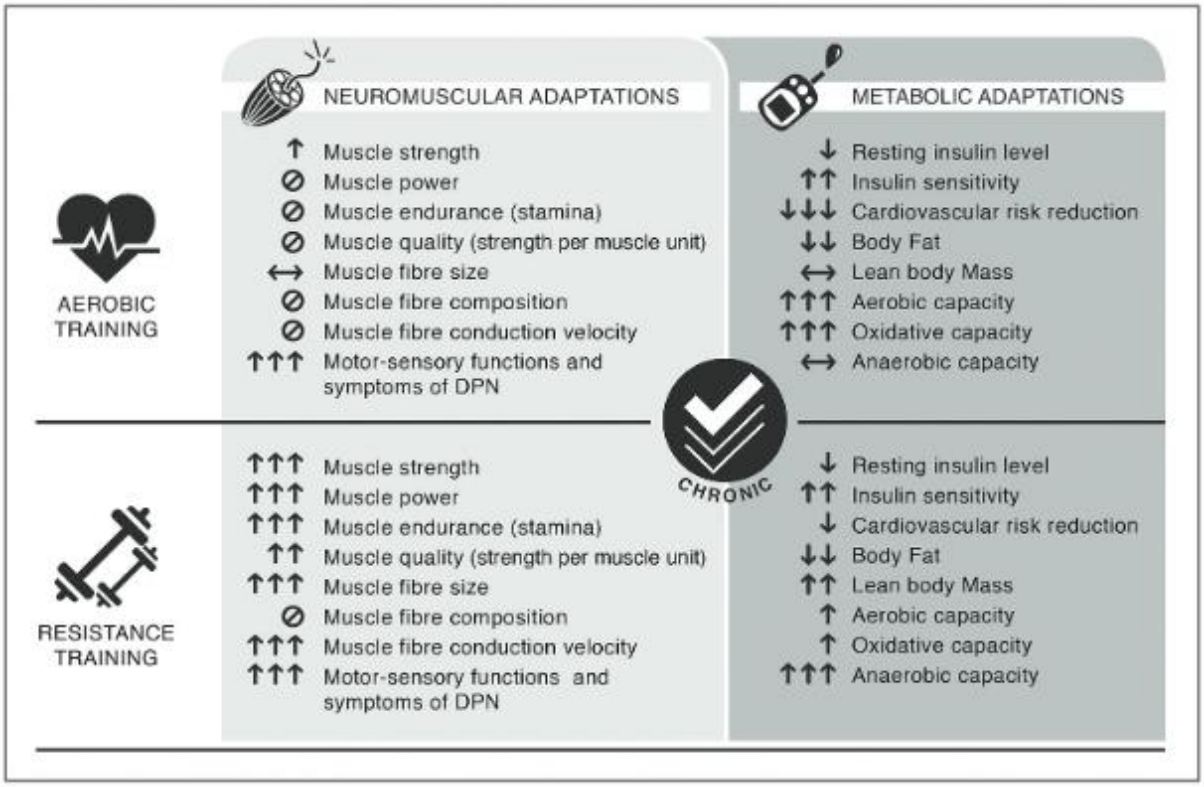

Figure 4 Basic neuromuscular and metabolic chronic modifications induced by aerobic and resistance training.
↔, parameter remain unchanged; ↑, small increase; ↑↑, moderate increase; ↑↑↑, large increase; ↓, small decrease; ↓↓, moderate decrease; ↓↓↓, large decrease; Ø, insufficient evidence available; DPN, diabetic peripheral neuropathy (≈ dNP) [6].

The above-mentioned findings are the foundation for the recently published ADA guidelines, who recommend aerobic exercises with moderate to vigorous intensity at a frequency of 3-7 days a week, in combination with gradually performed resistance

training. These resistance exercises are recommended for both the treatment of T2DM and for the prevention and management of sarcopenia. In addition to these two training modalities, the ADA guidelines also recommend flexibility and balance training, as these exercises are also important e.g. to attenuate limited joint mobility in the prevention of falls and accidents [51, 59].

Concerning our target population, T2DM patients with dNP, there is a paucity of studies that examine the effect of aerobic and/or resistance training on the prevention of dNP or potential reduction of the severity of dNP, on neuropathic pain and other QoL factors as endpoint measures [60]. Actually, aerobic exercise results in beneficial effects on many of the pathways that are adversely affected by T2DM. Moreover, it can improve three of the biggest risk factors for dNP: insulin sensitivity and glucose control, obesity and dyslipidemia [60]. With respect to the above recommended combined training program, T2DM patients with dNP should ideally follow an 8-12 weeks aerobic training program ((optionally home-based) walking, jogging, running, cycling, …), 3x/week during 20-45 minutes [61, 62], in combination with a progressive resistance exercise training program as this intervention program may increase skeletal muscle strength, muscle size and functional capacity in older adults [58, 60, 63, 64]. Some researchers described potential different phases in the building up of an resistance training program with emphasis on the major muscle groups of the different body parts (chest, back, arms, shoulders, upper legs and lower legs):

- Beginner: familiarization phase in order to ameliorate neuromuscular coordination,
  - Phase I 50-60% 1 repetition maximum (RM); 2x/week; 1-2 weeks; 1 set; 12-15 repetitions (reps),
  - Phase II 60-69% 1RM; 2x/week; 3-8 weeks; 1 set; 12-18 reps;
- Intermediate: emphasis on muscle endurance,
  - Phase I 60-69% 1RM; 2x/week; 9-16 weeks; 2 sets; 12-18 reps,
  - Phase II 70-79% 1RM; 3x/week; 17-24 weeks; 2 sets; 10-15 reps;
- Advanced: emphasis on hypertrophic effect on skeletal muscle fibers,
  - Phase I >80% 1RM; 3x/week; 25-32 weeks; 2 sets; 8-12 reps,
  - Phase II >80% 1RM; 4x/week split; 32+ weeks; 3 sets; 6-10 reps [58, 63].

Yet, it must be mentioned that guidance and monitoring by exercise experts, such as physical trainers and physiotherapists, are crucial to make appropriate patient-tailored adjustments (e.g. with regard to blood pressure control). Under all circumstances,

especially when older people are involved, injury prevention and the patient's motivation play a crucial role in training adherence. Therefore, supervised therapeutic exercise strategies are preferable as better results are reported compared to unsupervised aerobic or resistance exercises [60, 65].

It must be highlighted that longitudinal research is requested to explore and even adjust both training programs according to the patients' needs with differentiation between patients without dNP, and patients with dNPs or dNPsm. When taking into account the results of this dissertation, T2DM patients without dNP could benefit more from an in-depth training of neuromuscular coordination and an aerobic exploration of the peripheral muscles, which could be extended in consideration of the resistance training component. Contrary, T2DM patients with dNP, but in particular those with dNPsm, could also benefit from combined training sessions, albeit with special focus on the resistance training component with regard to hypertrophy of the muscle fibers to maximally strengthen the peripheral skeletal muscles of lower and upper limbs and to optimize explosive muscle strength.

Last but not least, as there is a more pronounced impact of dNP on the distal compared to proximal muscles in the lower limb, particular attention must be given to strengthen up the ankle joint as much or even more than the knee joint in function of fall risk prevention. The same applies to the upper limb, albeit to a lesser extent, with more emphasis to the strengthening of the hand muscles compared to the elbow in function of ADL, functionality, independence, housekeeping, getting dressed, etc.. Actually, 'functional' or 'task specific' exercises (such as climbing stairs, reaching something above the head, get up from a chair, lunges, …) have merit for enhancing physical function in the older population who are not yet disabled, as this type of training reduces the need for task modification of ADL [58]. Furthermore, the entire ageing population, including the patients with T2DM, could even benefit from unstructured physical activities, which are essential for reducing the number of consecutive sedentary hours, and, thereby, reducing the total time spent seated [51].

4. Future research perspectives

Until now, diabetes mellitus (DM) has been classified into two main forms: type 1 (T1DM) and T2DM. This classification, based on ADA criteria, primarily relies on the presence (T1DM) or absence (T2DM) of autoantibodies against the pancreatic islet β-cells antigens and on the patient's age at diagnosis. However, the predominant T2DM

variant is highly heterogeneous with regard to clinical presentation and progression. As this ADA-grounded diagnosis was only based on the measurement of only one metabolite (i.e. glucose), Swedish [66] and German [67] researchers considered *a novel diabetes classification* in patients with newly diagnosed DM by metabolically comparing six commonly measured variables (routine blood sampling, estimated glomerular filtration rate, insulin secretion and sensitivity, peripheral nerve function, autonomic nerve function, and eye conditions). Subsequently, five replicable clusters were withheld: mild age-related DM, mild obesity-related DM, severe autoimmune DM, severe insulin-resistant DM, and severe insulin-deficient DM [66]. In the knowledge that dNP is a prevalent disabling disorder with a wide pattern of symptoms, associated with a broad spectrum of risk factors, it could be of utmost importance to assign this complication to one of this new substratification within T2DM. A preliminary study of Zaharia et al., 2019, indicated that insulin deficiency or hyperglycemia are the most important triggers of dNP, confirmed by the highest recorded prevalence in patients with severe insulin-deficient DM [67]. Future research could benefit from this new stratification protocol for the allocation of patients with T2DM to the dNP+ group by focusing on this specific cluster.

Furthermore, all patients with T2DM, but in particular the above described severe insulin-deficient DM patient cluster, would benefit from the use of *sensitive diagnostic methods* for early detection and prediction of dNP, and prevention of major clinical outcomes. However, in patients without diagnosed dNP, the options of preventing dNP remain scarce and future controlled intervention trials are required in the detection of the predictive value and the possible efficacy of targeted treatment (medical therapy as well as physiotherapy). In patients who yet do suffer from dNP, the future research direction could be two-folded. First, it is well-known that the combination of aerobic exercise and resistance training improves blood glucose control, which is already a crucial benefit for patients with T2DM. Given that T2DM accelerates loss of muscle mass/strength with ageing (i.e. accelerated sarcopenia), the need to focus on resistance training arises. On top of this, the presence and severity of dNP causes an even greater and gradual loss of muscle strength, which accentuates the importance of muscle strength training even more. In the Chapter ‘Clinical implications’ an example of a progressive resistance exercise training program is written down, based on two articles [58, 63]. Secondly, it is of utmost importance to investigate the responsivity of this strength training in patients with dNPsm (i.e. the most affected diabetic subgroup)

compared to the patients without dNP (i.e. the best preserved group). The design of these future studies should rather be longitudinal and prospective.

Although ENMG (sensory and motor nerve conduction studies of large myelinated upper and lower limb nerves) is assumed to be superior to clinical neurological assessment tools for neural (dys)function, *corneal confocal microscopy* can assess the function of small neuron fibers instead of only large fibers. This technique could be of additional value as to indicate early onset of neuropathy. Patients with T2DM can already experience distant symptoms of neuropathy whilst ENMG data are still normal. These first clinical signs may be accompanied by muscular dysfunction, as written down by Almurdhi et al., 2017. They provided also evidence that small fiber abnormalities (e.g. corneal nerve fiber neuropathy) were related to reduced ankle plantar flexor strength in a population with impaired glucose tolerance [68].

At last, we found only three relevant studies exploring the effect of *inspiratory muscle training* (IMT) on patients with T2DM. Two researchers focused on patients with inspiratory muscle weakness (arbitrarily chosen cut-off (PI_{max} <70% predicted)) [69] and patients with autonomic cardiovascular dNP [70]. They randomly assigned these patients to eight weeks of IMT or eight weeks of placebo IMT. Both studies showed that IMT was effective in improving the strength of the inspiratory muscles (significant results even after two weeks [69]), but it did not improve pulmonary function and functional capacity [69, 70]. One particular study focused on three groups: patients without and with autonomic cardiovascular dNP, and HC [71] with significant lower PI_{max} and PI_{max} percentage predicted in both patient groups compared to HC. As in all studies mentioned above, the reference values for PI_{max} were based on older data [72], respiratory muscle weakness and the effect of in- and expiratory muscle training on T2DM patients without and with dNP warrants further investigation. Furthermore, future research on respiratory muscle training in those patients with a PI_{max} and/or PE_{max} below the more recent reference values of the specific age groups (for PI_{max} 50-59y, 60-69y, and 70-83y [73], and for PE_{max} 50-69y and >70y [74]) could provide more information on the possibility to strengthen the respiratory muscles by this kind of rehabilitation modality. The vast majority of physiotherapists specialized in respiratory rehabilitation already use portable powerbreathe devices in order to strengthen the respiratory muscles in patients with COPD, heart failure and diaphragmatic paresis [75]. However, recent research by use of these devices in patients with T2DM (without or with dNP) is scarce.

In addition, as adherence to exercise programs is a major point of attention in diabetic patients, future research assessing the lifestyle intervention factors that influence adherence to exercise programs is recommended [51]. The exercise expert (the physical trainer or the physiotherapist) should identify and focus on the individual beliefs of the patients rather than on general health benefits, by setting specific patient-tailored enjoyable goals and avoiding discouragement of unrealistic expectations. The compliance also relies on the patient's personality and will be affected by offering a group exercise class program for the one, and an individual home program for the other [76]. These needs could be questioned by means of a survey and could enhance the *adherence and compliance* to predetermined exercise programs.

First, in this dissertation we started up preliminary studies in order to detect and to face the actual impact of T2DM and dNP on respiratory and peripheral muscle strength. Nowadays, the focus on dNP directed physical activity programs can start (resistance training programs under others). Consequently, the ultimate aim -after finishing these preliminary studies- was/is/will be to set up a study protocol regarding directed physical activity programs for patients with T2DM, without and with dNP. However, in this set-up, extra attention has to be given to the *feasibility and safety* of these programs, as patients who do not suffer from any (serious) adverse event could start their training program at a higher level or the supervising specialist could increase the percentage of 1RM somewhat faster.

5. Final conclusions

All patients with T2DM, independently of the presence of dNP, experienced significantly lower maximal in- and expiratory pressure and peak expiratory flow measurements compared to the healthy controls. Furthermore, concerning muscle endurance total work in the lower limb of the same entire patient group, a reduction was observed for knee extension/flexion (20-30%) and ankle plantar/dorsiflexion (50-60%), whilst the muscle endurance total work in the upper limb (elbow extension/flexion and handgrip strength) remained less affected.

In T2DM patients with clinically diagnosed dNP (no in-depth investigation), significantly lower maximal inspiratory pressure measurements were found compared to the healthy controls, which may be explained by the extra muscular effort during inspiration, debilitated by the presence (and probably the severity) of dNP.

T2DM patients with the worst type of dNP (patients with sensorimotor dNP) showed significantly reduced explosive strength and maximal lower limb muscle strength compared to the healthy controls. Hence, these results indicate a deteriorating effect on explosive and maximal muscle strength due to the presence and severity of dNP. Concerning maximal lower limb muscle strength ratios, the ankle strength was significantly more reduced compared to the strength of the knee, dependent on the presence and severity of dNP. Regarding the upper limb, no significant results could be reported, indicating a more pronounced distal-to-proximal impact of dNP in the lower limb compared to the upper limb.

In future studies, exercise experts, such as physical trainers and physiotherapists, should focus on the development of patient-tailored exercise programs (in function of the presence (and severity) or absence of dNP) with special emphasis on constant patient-motivation and conscientious adherence to the patient-adjusted training scheme.

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PART IV

ENGLISH SUMMARY
NEDERLANDSTALIGE SAMENVATTING

1. English summary

Type 2 diabetes mellitus (T2DM), accounting for around 90% of all diabetes cases, is a chronic metabolic disease which has nowadays reached proportions of a pandemic (425 million people worldwide and estimated to rise to 629 million by the year 2045).
Diabetic neuropathy (dNP) is a frequent complication of T2DM with motor dysfunction as a late and severe manifestation. Consequently, in order to sustain daily functioning, walking speed, physical performance, and to maintain or even enhance the quality of life in patients with T2DM, a solid and innovative physiotherapy approach is of our interest.
The core aim of this dissertation was to gain more insight in the detrimental effects of dNP on the peripheral skeletal muscle strength in patients with T2DM.
PART I of this dissertation (general introduction and outline/scope) focuses on normal glucose homeostasis and mechanisms of insulin action in healthy subjects and on the pathophysiology in patients with T2DM. The gold diagnostic criteria, standard treatment plan and the prevention of diabetic comorbidities are considered, based on the current guidelines and recommendations. When closely examining the T2DM population, we were particularly interested in patients with dNP, as this disorder may impact these patients' level of physical activities, their gait, balance and coordination, their functional independence and, last but not least, their overall quality of life.
In the early stages of dNP, patients often present with predominant involvement of the sensory nervous system (sensory symptoms) without detectable clinical motor impairment. After years of dNP exposure and progression in patients with T2DM, approximately 50% of the sensory dNP evolves into sensorimotor dNP with clinical impairment in motor function and atrophy of the skeletal muscles in the end.
PART II (original research) exclusively concentrates on the impact of dNP on respiratory muscle strength, skeletal maximal and explosive muscle strength and on muscle endurance in patients with T2DM.
First, we investigated the influence of clinically diagnosed dNP on respiratory muscle strength in patients with T2DM (n=110) compared to 35 healthy controls. The results of this study revealed significantly lower maximal expiratory pressure and peak expiratory flow measurements in all patients with T2DM, independently of the presence of dNP compared to the healthy controls. Concerning maximal inspiratory pressure, the only significant differences were found between the T2DM patients with clinically

diagnosed dNP (n=82) and the healthy controls, maybe due to the extra muscular effort during inspiration which could be debilitated by the presence of dNP.

Secondly, we discussed the impact of sensory and/or sensorimotor neuropathy on lower limb muscle endurance, explosive and maximal muscle strength in patients with T2DM. In this study, a reduction in lower limb muscle endurance was observed by means of lower levels for total work in knee extension/flexion (20–30%) and ankle plantar/dorsiflexion (50–60%) in all patients with T2DM (without dNP (n=8), as well as with sensory (n=13) and sensorimotor dNP (n=14)) compared to healthy controls (n=19). Both explosive and maximal muscle strength were significantly reduced in the worst dNP group (patients with sensorimotor dNP) compared to the healthy controls. Hence, the results of this study indicate a deteriorating effect on explosive and maximal muscle strength due to the presence of the most severe (sensorimotor) dNP, while T2DM as such predominantly affects muscle endurance.

The last study, also cross-sectionally designed, was conducted to investigate the impact of dNP on the distal versus proximal comparison of weakness in the lower and upper limb muscles of patients with T2DM. Analysis showed that muscle endurance total work revealed significantly reduced ankle data compared to the knee, and, again, independently of the presence of dNP. Concerning maximal muscle strength, the ankle data were significantly more reduced compared to knee data, which was dependent on the presence and severity of dNP as the decrease was most pronounced in patients with sensorimotor dNP. Regarding the upper limb, no significant differences could be reported between elbow and hand within the three diabetic groups. The above mentioned results may indicate a more pronounced distal-to-proximal impact of dNP in the lower limb compared to the upper limb.

Finally, **PART III** (general discussion and future research) reflects on the clinical implications regarding a possible analytical training program and some functional training aspects, on the strength and limitations of the conducted research, and on the future research perspectives.

Final conclusions:

Compared to healthy controls,

all patients with T2DM (without or with dNP) showed

- significantly lower maximal expiratory pressure and peak expiratory flow measurements, independently of the presence of dNP,
- a muscle endurance total work reduction in ankle plantar/dorsiflexion (50–60%) and in knee extension/flexion (20–30%),
- no significant muscle endurance total work differences in elbow extension/flexion and handgrip strength.

T2DM patients with clinically diagnosed dNP (by means of clinical neurological examination) yielded

- significantly lower maximal inspiratory pressure measurements may be due to the extra muscular effort during inspiration which could be debilitated by the presence (and probably the severity) of dNP on this extra muscular effort.

T2DM patients with the worst type of dNP (sensorimotor dNP) revealed

- significantly reduced explosive strength and maximal ankle and knee muscle strength, indicating a deteriorating effect due to the presence and severity of dNP,
- no significant differences between maximal handgrip and elbow strength, indicating a more pronounced distal-to-proximal impact of dNP in the lower limb compared to the upper limb.

In future studies, exercise experts, such as physical trainers and physio-therapists, should focus on the development of T2DM patient-tailored exercise programs (in function of the presence (and severity) or absence of dNP) with special emphasis on constant patient-motivation and conscientious adherence to the patient-adjusted training scheme.

2. Nederlandstalige samenvatting

Negentig procent van alle diabetespatiënten lijdt aan type 2 diabetes mellitus (T2DM), een chronische metabole aandoening die de proporties van een pandemie bereikt (vandaag 425 miljoen mensen wereldwijd met een geschatte stijging tot 629 miljoen tegen 2045). Diabetische neuropathie (dNP) is een veelvoorkomende complicatie bij T2DM- patiënten met motorische dysfunctie als een late en ernstige manifestatie. Bijgevolg behoort het behoud van dagelijks functioneren, stapsnelheid, fysiek functioneren, het onderhouden en eventueel verbeteren van de levenskwaliteit tot één van de belangrijkste doelstellingen. Dit kan bereikt worden door middel van een meer doelgerichte kinesitherapeutische aanpak.

Dit doctoraal proefschrift heeft als doel de aandacht te vestigen op de nadelige invloed van dNP op de perifere spierkracht bij patiënten met T2DM.

DEEL I van dit doctoraal proefschrift (algemene inleiding en onderzoeksvragen) legt de focus op de glucose homeostase en de werking van insuline bij gezonde mensen en op de pathofysiologie bij patiënten met T2DM. De momenteel geldende diagnostische criteria, het standaard behandelplan en de preventie van ziektegerelateerde comorbiditeiten worden hier aangehaald. Gezien de totale T2DM-populatie reeds goed bestudeerd werd, hebben wij besloten om ons voornamelijk te richten op de dNP-groep gezien de mogelijke impact op hun fysieke activiteit, evenwicht en coördinatievermogen, functionele onafhankelijkheid en, boven alles, hun algemene levenskwaliteit.

In de beginfase van dNP wordt het sensorisch zenuwstelsel voornamelijk aangetast (sensorische symptomen) zonder klinisch waarneembare motorische aftakeling. Pas na jaren blootstelling aan en evolutie van dNP, ontwikkelen 50% van deze patiënten sensorimotorische klachten met klinisch waarneembare motorische dysfunctie, waaronder finaal atrofie van de skeletspieren.

In **DEEL II** (origineel onderzoek) wordt de nadruk gelegd op de impact van dNP op de respiratoire spierkracht, de maximale en explosieve skeletspierkracht en de spierkracht-uithouding.

In de eerste studie werd de invloed van klinisch gediagnosticeerd dNP op de respiratoire spierkracht onderzocht bij patiënten met T2DM (n=110) en 35 gezonde vrijwilligers. De resultaten toonden aan dat de maximale expiratiedruk en de maximale luchtstroomsnelheid bij alle T2DM-patiënten, onafhankelijk van de aanwezigheid van

dNP, significant lager waren vergeleken met de gezonde vrijwilligers. Significant lagere maximale inspiratiedrukwaarden werden enkel gevonden bij die patiënten met klinisch gediagnosticeerde dNP (n=82) in vergelijking met de gezonde vrijwilligers, hetgeen zou kunnen te wijten zijn aan de extra spierinspanning tijdens het inademen en de negatieve invloed van dNP (aanwezigheid en eventuele ernst) op de skeletspierkracht. De tweede studie werd opgesteld om de impact van sensorische en sensorimotorische dNP op de spierkracht-uithouding, explosieve en maximale spierkracht te analyseren in de onderste ledematen van patiënten met T2DM. Hier werd een verminderde spierkracht-uithouding geregistreerd voor wat betreft de maximaal geleverde arbeid gedurende één beweging (= 'total work') voor plantair/dorsiflexie (50–60%) en voor knie extensie/flexie (20–30%) in alle patiënten met T2DM (zowel zonder dNP (n=8), als met sensorische (n=13) en sensorimotorische dNP (n=14)) in vergelijking met de gezonde vrijwilligers (n=19). Bij de explosieve en maximale spierkracht analyse zagen we significant lagere waarden in de patiëntengroep sensorimotorische dNP, vergeleken met de gezonde vrijwilligers. Bijgevolg wijzen deze resultaten aan dat de aanwezigheid en de ernst van dNP een extra negatief effect heeft op explosieve en maximale spierkracht in de onderste ledematen van patiënten met T2DM, terwijl de ziekte op zich reeds voldoende is om een verminderde spierkracht-uithouding te veroorzaken.

In de derde transversale studie werd een vergelijking gemaakt van de impact van dNP op de distale versus proximale spierkracht in patiënten met T2DM. Analyse toonde bij spierkracht-uithouding 'total work' lagere enkelwaarden vergeleken met de kniewaarden en dit opnieuw voor alle patiënten met T2DM, ongeacht de aanwezigheid van dNP. De maximale spierkracht data van de enkel waren significant lager dan die van de knie bij de patiëntengroep met sensorimotorische dNP, duidend op de impact van dNP op deze krachtparameter. Er werden geen significante verschillen gevonden tussen handknijp- en elleboogkracht in geen van de drie onderzochte diabetesgroepen, wijzend op een meer uitgesproken distaal-naar proximaal verloop van dNP in het onderste lidmaat vergeleken met het bovenste.

DEEL III (algemene discussie en toekomstig onderzoek), het laatste deel, bood ons de ruimte om te reflecteren over de klinische implicaties met een toekomstgerichte maar ruwe schets van een analytisch trainingsplan en ook de functionele trainings-aspecten, een sterkte-zwakte analyse van het gevoerde onderzoek en specifieke perspectieven voor onderzoek in de toekomst.

Eindconclusie:

Vergeleken met de gezonde controlegroep,

toonden alle patiënten met T2DM (zonder of met dNP)

- significant lagere maximale expiratiedruk en maximale luchtstroomsnelheid metingen, onafhankelijk van de aanwezigheid van dNP,
- een verminderde enkel plantair/dorsiflexie van 50 tot 60% en een verminderde knie extensie/flexie van 20 tot 30% bij spierkracht-uithouding 'total work',
- geen significante verschillen tussen handgrijp- en elleboogkracht bij spierkracht-uithouding 'total work'.

vonden we bij T2DM-patiënten met klinisch gediagnosticeerde dNP (aan de hand van klinisch neurologisch onderzoek)

- significant lagere maximale inspiratiedruk metingen enkel in die patiëntengroep met klinisch gediagnosticeerde dNP, hetgeen zou kunnen te wijten zijn aan de extra spierinspanning tijdens het inademen en aan de negatieve invloed van de aanwezigheid (en eventuele ernst) van dNP op deze extra spierinspanning.

hadden T2DM-patiënten met de ernstigste vorm van dNP (sensorimotorische dNP),

- significant verlaagde explosieve kracht en maximale spierkracht in enkel en knie, wijzend op een extra negatief effect van de aanwezigheid en de ernst van dNP,
- geen significante verschillen tussen maximale handknijp- en elleboogkracht, hetgeen wijst op een meer uitgesproken distale-naar-proximale impact van dNP in het onderste lidmaat in vergelijking met het bovenste.

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- bureau 10: Julie, Anke, Bieke, Cynthia, Gert… mijn eerste AAP jaren onder Dirk
- bureau 11: Ian, Dorien, Cajsa, Karsten, Anouk, Hellen, Marie, Bert, Eric, … mijn eerste AAP jaren met 'de ramen toe' onder Patrick
- bureau 12: Karsten, Hellen, Bert/Jan… en nu met 'de ramen wagenwijd open'!
- KO BL-vriendjes: Annelies, Birgit, Ann, Cedric, Aurélie, Paul, Bieke, Ziggy, Vanessa, Marie, Fran, Camilla, Dries, Kevin, Brecht, Dominiek, …
- Dienst Urologie-vriendjes: Catherine en Johan, Piet en Roberto, Brigitte, Jean-Jacques, …
- RevaSKI-vriendjes: Barbara, Nicolas, Ann, Rudi, Mira, Umit, Lieven, Erik, Hilde, Bruno, Narmin, Cedric, Koen, Dorien, Roel, Willem, Aurélie, Kayleigh, Thibault, Sandra, Stijn, Paolo, Gert, Vanessa, Karsten, Jente, Thierry, Sabine, Hans, Dirk, Vincent, Changiz, Wiene, …
- Barbara, Evi, Sara, Kayleigh, Sandra, Kathleen, Mira, …: voor jullie persoonlijke interesse en onverwachte oppeppers. Jullie zitten voor eeuwig in mijn hart…
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- Leen, Els, Valérie, Eve, Ans, Brecht, William, Samuel, Niels, Tim, Koen, Tom, …

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DANK AAN mijn familie:

- Opa Gaby en Oma Jeanneke, mijn ouders met hun onvoorwaardelijke liefde en onnavolgbaar geduld voor hun o-zo'n zootje ongeregelde kroost… Maar dan ook '*special thanks to my mom*©' gewoon en heel simpel omdat ze is wie ze is…)
- Oma Ria en Emmanuel†, mijn schone mama met haar uitzonderlijk warm karakter en jullie legendarische 'dikke knuffel(s)'… (en onze inside-grapjes…)
- Mijn broers en zussen (Peter, Greet, Catharina en Karl), hun '*partners in crime*' (An, Hans, Pascale, …) en hun talrijke nakomelingen (Viktoria!). *Special thanks to Catharina*®, die me bij tijd en stond bij de hand nam, me zo nodig eens deftig dooreen schudde ('*shaken PhD student syndrome*') en me terug via Schleiper, CRU en de BEO-markt naar het rechte pad leidde… èn mijn cover ontwierp!
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En dan nu, **DANK AAN mijn 'ALLES'**:

- De steeds verrassende Jérôme, sterke persoonlijkheid en blakend van zelf-vertrouwen op amper 23 jarige leeftijd, wetend wat hij wil en waar hij naartoe leeft (ook al is het voor ons momenteel niet helemaal duidelijk welke weg hij nu zal bewandelen). Een zoon die via Google zijn moeder haar 'bekendheid' opzocht om dan strijk achterover te vallen hoeveel hits er verschenen…
- De onzekere Simon, 22 jaar, twijfelachtig zoekend naar die paden die hij zou willen bewandelen, dromend en hopend op weg naar steeds opnieuw de juiste keuzes, een hobbelig parcours naar een mooie toekomst vol verrassingen en uitdagingen.
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- *Last but not least*, het 'Kasper'nestje van de familie, 19 jaar met adonis-achtige looks, toekomstgerichte visie, verborgen maar ontluikende capaciteiten, leergierig maar ook zeer zelfbewust, onzeker en zelfzeker tegelijkertijd, dankbaar maar toch veeleisend, altruïstische trekjes met toch dat vleugeltje narcisme, soms nog wat jong van geest maar juist net door die jeugdigheid o-zo geestig …
- En dan… we kunnen er niet onder uit, we kunnen niet rond de pot blijven draaien, we moéten doorbijten: Brat met Pitt! Lieve Bart, kan je alle mensen nog op twee handen tellen die je ooit vroegen hoe je het kon/kan/zal blijven uithouden met een ADHD belaste, autismespectrum beladen, revolterende en rebelse, contraire, zwart-wit denkende, steeds denkende, nadenkende, nooit-loslatend denkende, anders denkende persoon naast je zijde (terwijl jij eigenlijk de moeilijke bent in onze relatie… 😉)? Lieve Bart, kan je je überhaupt één dag voorstellen zonder dit veelzijdig persoontje naast je zijde? Ik dacht het niet… (Je hebt dus niet écht een keuze, *I know*…). En toch moet ik toegeven, al ligt dit enigszins moeilijk, dat ik hier niet zou geraakt zijn zonder jou, je geloof in mijn kunnen, het vertrouwen in mijn verwarde geest en de liefde voor mijn afgetakeld basketlijf… Een welgemeende

MERCI voor hetgeen je reeds voor me gedaan hebt, doet en hopelijk zal blijven volhouden tot het bittere eind.

Al jullie namen zullen geprezen zijn!

APPENDICES

1. Curriculum vitae

Personal details

Name: Birgitta, Lutgard, Marcella Van Eetvelde
Date of birth: July 4th, 1969
Place of birth: Ghent, Belgium
Mobile phone: +32 479 27 95 81
E-mail: vaneetveldebirgit@gmail.com

Education

2015 – 2021	PhD student in Health Sciences, Department of Rehabilitation Sciences (Diabetic neuropathy), Ghent University, Belgium
1993 – 1995	PhD student in Health Sciences, Department of Motoric Revalidation and Physiotherapy (Pelvic floor rehabilitation), University of Antwerp, Belgium
1991 – 1993	PhD student in Health Sciences, Department of Motoric Revalidation and Physiotherapy (Pelvic floor rehabilitation), Ghent University, Belgium
1987 – 1991	Licentiate in the Motoric Revalidation and Physiotherapy Ghent University, Belgium
1981 – 1987	Sint-Bavo Humaniora, Latin-Sciences Ghent, Belgium

Professional experience

2015 – 2017	Geriatric physiotherapist in a Residential Care Setting 'Avondvrede' in Ghent
2013 – 2015	Scientific Assistant at the Department of Rehabilitation Sciences Ghent University, Belgium
2004 – 2013	Medical delegate for Schering-Plough / Merck Sharp & Dohme Pharmaceutical Company in Brussels
2001 – 2004	Health Care Associate for AstraZeneca Pharmaceutical Company in Brussels
2000 – 2001	Quality Coordinator at the Drug Research Unit Ghent (D.R.U.G.) Ghent University Hospital, Belgium
1997 – 2000	Clinical Research Associate for Byk Belga Pharmaceutical Company in Brussels
1995 – 1997	Medical delegate for Leo Pharmaceutical Products Pharmaceutical Company in Brussels

Teaching experience

2015 – 2019 Physiological examination of the upper limb: Bachelor 1
Department of Rehabilitation Sciences, Ghent University, Belgium

2019 – 2021 Physiological examination of the upper limb: Bachelor 2
Department of Rehabilitation Sciences, Ghent University, Belgium

Publications

Pelvic floor rehabilitation:

- Comparison in Young Healthy Volunteers of 3 Different Parameters of Constant Current Stimulation used to determine Sensory Thresholds in the Lower Urinary Tract; J.J. Wyndaele, **B. Van Eetvelde** and D. Callens; Journal of Urology, Vol. 156, October 1996, pp. 1415-1417
- Reproducibility of Digital Testing of the Pelvic Floor Muscles in Men; J.J. Wyndaele and **B. Van Eetvelde**; Archives of Physical Medicine and Rehabilitation, Vol. 77, November 1996, pp 1179-1181

Diabetic neuropathy:

- The Influence of Clinically Diagnosed Neuropathy on Respiratory Muscle Strength in Type 2 Diabetes Mellitus; **B. Van Eetvelde**, D. Cambier, K. Vanden Wyngaert, B. Celie, P. Calders; Journal of Diabetes Research. Volume 2018 Nov 29; 2018:8065938.
- The impact of sensory and/or sensorimotor neuropathy on lower limb muscle endurance, explosive and maximal muscle strength in patients with type 2 diabetes mellitus; **B. Van Eetvelde**, B. Lapauw, P. Proot, K. Vanden Wyngaert, B. Celie, D. Cambier, P. Calders; Journal of Diabetes and Its Complications, 2020 Jun; 34(6): 107562.
- The impact of diabetic neuropathy on the distal versus proximal comparison of weakness in lower and upper limb muscles of patients with type 2 diabetes mellitus: a cross-sectional study; **B. Van Eetvelde**, B. Lapauw, P. Proot, K. Vanden Wyngaert, S. Helleputte, J. Stautemas, D. Cambier, P. Calders; accepted article in the Journal of Musculoskeletal and Neuronal Interactions, 2021 July.

Co-authorship:

- Reproducible Measurements of Muscle Characteristics Using the MyotonPRO Device: Comparison Between Individuals With and Without Paratonia; B. Van Deun, J.S.M. Hobbelen, B. Cagnie, **B. Van Eetvelde**, N. Van Den Noortgate, D. Cambier; Journal of geriatric physical therapy (2001) 2018 Oct/Dec;41(4):194-203.
- The relationship between glycaemic variability and cardiovascular autonomic dysfunction in patients with type 1 diabetes: A systematic review; S. Helleputte, T. De Backer, B. Lapauw, S. Shadid, B. Celie, **B. Van Eetvelde**, K. Vanden Wyngaert, P. Calders; Diabetes/metabolism research and reviews 2020 Jul;36(5):e3301.

Training, Symposia and Meetings

Doctoral training program:

Statistical analysis with the help of SPSS (Starters)

- Cluster: Research & Valorization
- Organization: Biostatistics Unit, University of Ghent
- Dates and venue: 4/09/2015, 11/09/2015, 18/09/2015, 25/09/2015 (9:00-12:30)

Presentation skills in English AY 2016-2017

- Cluster: Transferable Skills
- Organization: Ghent University Doctoral School
- Dates and venue: 15/02/2017, 22/02/2017, 8/03/2017,15/03/2017 (9:00 – 12:00)

Communication Skills Basics AY 2017-2018

- Cluster: Leadership & personal efficiency
- Organization: Ghent University Doctoral School
- Dates and venue: 14/2/2018, 21/2/2018 (9:00 – 17:00)

Negotiation Skill, a follow-up module of the 2 days program 'Communication Skills Basics'

- Cluster: Leadership & personal efficiency
- Organization: Ghent University Doctoral School
- Date and venue: 25/4/2018 (9:00 – 17:00)

Advanced Academic English: Conference Skills - Academic Posters AY 2017-2018

- Cluster: Communication Skills
- Organization: Ghent University Doctoral School
- Dates and venue: 20/4/2018, 27/4/2018 (13:30 – 13:30)

Advanced Academic English: Writing Skills AY 2018-2018

- Cluster: Communication Skills
- Organization: Ghent University Doctoral School
- Passed the orientation test on 25/9/2018
- Dates and venue: 01/10/2018, 08/10/2018, 15/10/2018, 22/10/2018, 05/11/2018, 12/11/2018, 19/11/2018, 26/11/2018, 03/12/2018, 10/12/2018 (11:00 – 13:00)

University Language Center:

Lecturing Skills in English AY 2019-2020

- Application for cluster recognition: Transferable Skills Seminars
- Organization: Ghent University
- Dates and venue: 07/02/2020, 14/02/2020, 21/02/2020, 28/02/2020, 06/03/2020, 13/03/2020, 27/03/2020, 03/04/2020 + extra zoom session on 29/05/2020 (09:00 – 11:30)

Research Days & Student Research Symposia of the faculty of Medicine and Health Sciences, the faculty of Pharmaceutical Sciences and Ghent University Hospital:

- 19/04/2018
  Presentation of preliminary results of 'The influence of clinically diagnosed neuropathy on respiratory muscle strength in patients with T2DM.'

Metabolic Research Meetings:

- **organized by Prof. Dr. B. Lapauw, Ghent University Hospital (9K12):**
  09/11/2018
  Presentation of preliminary results of 'The impact of sensory and/or sensorimotor neuropathy on lower limb muscle endurance, explosive and maximal muscle strength in patients with type 2 diabetes mellitus.'
- **organized by Prof. dr. P. Calders, Ghent University (1B3):**
  28/11/2019, 06/03/2020, 03/04/2020, 08/05/2020, 12/06/2020, 04/09/2020, 22/10/2020, 16/11/2020, 18/12/2020 and 25/01/2021

CONGRESSES

54TH EASD ANNUAL MEETING IN BERLIN, FROM 1 - 5 OCTOBER 2018:
The abstract, titled "The influence of clinically diagnosed neuropathy on respiratory muscle strength in type 2 diabetes" (Submission-Number: A-18-978-EASD),accepted as a **Poster Presentation.**

55TH EASD ANNUAL MEETING IN BARCELONA, FROM 16 - 20 SEPTEMBER 2019:
The abstract, titled "The influence of diabetic neuropathy on muscle strength (maximal strength, strength-endurance and explosive strength) in patients with T2DM" (Submission-Number: A-19-931-EASD),accepted as a **Poster Presentation**.

56TH EASD ANNUAL MEETING WHICH WOULD BE HELD IN VIENNA, BUT DUE TO COVID-19 WILL BE HELD VIRTUALLY FROM 21 - 25 SEPTEMBER 2020:
The abstract, titled "Diabetic neuropathy impacts upper and lower limb muscle strength endurance in patients with type 2 diabetes: a controlled study" (Submission-Number: A-20-1842-EASD), accepted as a **Poster Presentation**.

2. Authors contribution

The influence of clinically diagnosed neuropathy on respiratory muscle strength in type 2 diabetes mellitus; Journal of Diabetes Research

Van Eetvelde B., Cambier D. and Calders P. conceived and designed the study, analyzed data, wrote, edited, and reviewed the manuscript.

Van Eetvelde B., Vanden Wyngaert K. and Celie B. researched data, contributed to the discussion and interpretation of data, and edited and reviewed the manuscript. All authors gave final approval for publication.

Van Eetvelde B. and Calders P. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Brief statements of assistance: we gratefully acknowledge Gysel T. for support in data collection.

The impact of sensory and/or sensorimotor neuropathy on lower limb muscle endurance, explosive and maximal muscle strength in patients with type 2 diabetes mellitus; Journal of Diabetes and its complications

Van Eetvelde B., Cambier D. and Calders P. conceived and designed the study, analyzed data, wrote, edited, and reviewed the manuscript.

Van Eetvelde B., Lapauw B., Proot P., Vanden Wyngaert K. and Celie B. researched data, contributed to the discussion and interpretation of data, and edited and reviewed the manuscript. All authors gave final approval for publication.

Van Eetvelde B. and Calders P. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Brief statements of assistance: we gratefully acknowledge Toye K. for assisting in the DXA and pQCT evaluations, and Gysel T. for support in data collection.

The impact of diabetic neuropathy on the distal versus proximal comparison of muscle weakness in the lower and upper limb of patients with type 2 diabetes mellitus: a cross-sectional study; Journal of Musculoskeletal and Neuronal Interactions

Van Eetvelde B., Cambier D. and Calders P. conceived and designed the study, analyzed data, wrote, edited, and reviewed the manuscript.

Van Eetvelde B., Lapauw B., Proot P., Vanden Wyngaert K., Helleputte S. and Stautemas J. researched data, contributed to the discussion and interpretation of data, and edited and reviewed the manuscript. All authors gave final approval for publication.

Van Eetvelde B. and Calders P. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Brief statements of assistance: we gratefully acknowledge Toye K. for assisting in the DXA evaluations, and Gysel T. for support in data collection.

East is East and West is West, and never the twain shall meet.

-Rudyard Kipling (1865-1936)-
The Ballad of East and West