

**MScanFit motor unit number estimation and muscle velocity recovery cycle recordings in diabetic polyneuropathy**

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## Abstract

**Objective:** Motor Unit Number Estimation (MUNE) methods may be valuable in tracking motor unit loss in diabetic polyneuropathy (DPN). Muscle Velocity Recovery Cycles (MVRCs) provide information about muscle membrane properties and can reveal disease-related changes. We aimed in this study to examine the applicability of the novel MUNE method MScanFit MUNE in anterior tibial muscle and to test whether the MVRCs could improve understanding of DPN pathophysiology.

**Methods:** Seventy-nine type 2 diabetic patients were compared to 32 control subjects. All participants were examined with the MScanFit MUNE and MVRCs in anterior tibial muscle. Lower limb nerve conduction studies (NCS) were applied to all patients to diagnose neuropathy.

**Results:** NCS confirmed DPN for 47 of the diabetic patients (DPN+), with 32 not qualifying (DPN-). MScanFit showed significant motor unit loss and increased motor unit sizes, when comparing DPN+ patients with controls, and also when comparing DPN- patients with controls, MVRCs did not differ between groups.

**Conclusions:** MScanFit MUNE reveals motor unit loss more sensitively than NCS in type 2 diabetic patients, whereas MVRCs do not provide additional information.

**Significance:** The MScanFit results show that the common description of DPN as primarily sensory is misleading, and may have distracted attention from role of the axonal environment in the pathophysiology of DPN.

Keywords: Diabetic polyneuropathy; MScanFit; Motor unit number estimation; Muscle velocity recovery cycles; motor unit loss

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## 1. Introduction

According to the World Health Organization, diabetes has reached epidemic status. The result is an increase of type 2 diabetics to an estimated 693 million by the year 2045 (Cho et al., 2018). Type 2 diabetic patients have an increased risk of a number of co-morbidities – one of which is neuropathy.

The most common type of neuropathy in diabetic patients is the length-dependent symmetrical neuropathy. In this type, the neuropathy starts in the most distal part of the lower extremity and continues proximally. The upper extremity is involved in the later stages of disease and develops in the same manner (Pop-Busui et al., 2017).

The patients mainly report sensory symptoms, but in the later stages, motor symptoms are also prevalent (Feldman et al., 2017). Diabetes is often assumed to target sensory axons preferentially due to the symptomology (Feldman et al., 2019). However, in a previous study we have shown that motor nerve fibers have signs of degeneration as early as sensory changes in the upper limbs. While conventional nerve conduction studies (NCS) could not reveal any motor abnormality, probably due to collateral sprouting and reinnervation by motor fibers, a novel motor unit number estimation (MUNE) method, MScanFit MUNE, has revealed motor abnormalities similar to the sensory ones in the median nerve (Kristensen et al., 2019a). MUNE encompasses several methods of assessing the number of motor units in the examined muscle. Loss of motor units is of interest in many diseases to evaluate severity and track progression. The newest method, MScanFit MUNE, fits a stochastic model to a compound muscle action potential (CMAP) scan (Bostock, 2016). Our previous findings of a decreased number of motor units in the upper extremity of type-2 diabetic patients with neuropathy (Kristensen et al., 2019a), suggested that the extent of motor nerve involvement in diabetic neuropathy may have been underestimated, and raised the need to re-evaluate the changes in the lower extremity.

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Nerve conduction studies and MUNE methods provide information on the extent of nerve degeneration, but are unable to give further insights to the mechanisms behind the degeneration.

Muscle velocity recovery cycles (MVRCs) provide a method of assessing changes in the membrane properties of muscle fibers – analogous to the use of excitability recovery cycles to detect changes in the membrane properties of axons. For example, the supernormality (period of increased conduction velocity) following a muscle action potential resembles the superexcitability in axons in providing a sensitive indicator of changes in membrane potential (Z'Graggen & Bostock, 2009). Furthermore, Tan and colleagues found changes in chloride and sodium channel myotonias that demonstrate the ability of MVRCs to detect the functional consequences of different channel defects compared with the healthy controls (Tan et al., 2014; Tan et al., 2018). We have previously reported *in vivo* evidence of depolarization in neurogenic muscles using MVRCs (Witt et al., 2019) which provided insights to the pathophysiology of these conditions. The present study is the first to apply MVRC recording to diabetic patients.

Our aim in the present study was to investigate if our previous findings of motor nerve degeneration from the upper limb of type-2 diabetic patients with neuropathy translated to the lower limbs, and to explore if the muscle membrane excitability could reveal more about the underlying mechanisms behind diabetic polyneuropathy (DPN).

## 2. Methods

### 2.1. Participants

Participants for this study consisted of type 2 diabetics recruited as part of another study on diabetes, fall risk and strength training. They were recruited from the DD2 Cohort and the outpatient clinic of the Endocrinology Department. Details on recruitment are provided elsewhere (Ref Karolina's study). In the present study, 96 patients were included. Seventeen patients were

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excluded due to inability to reach supramaximal stimulation and discomfort in MScanFit MUNE studies, when stimulating at the lower limb. Thirty-three age and sex-matched controls were included, one could not tolerate the examinations and was excluded.

## 2.2. Equipment

Recording and analysis of recordings are handled by two parts of the same software package, QtracW (Digitimer Ltd). For both MScanFit and MVRC's the equipment is similar, except for the protocols and electrodes used. Output is controlled by the QtracS part of QtracW on a laptop connected to a DS5 stimulator (Digitimer Ltd) with leads to the stimulating electrodes or needle. The recorded signals are sent to a D440 amplifier (Digitimer Ltd) with recording electrodes or needles. The amplified signal is filtered through a HumBug 50 Hz noise eliminator (Quest Scientific Inc.) and input back to the laptop and QtracS. Signals are converted between analog and digital with the National Instruments USB-6221 BNC. The setup is pictured in figure 1.A.

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## 2.3. MScanFit MUNE

For MScanFit measurements, stimulation was via adhesive surface electrodes. The cathode was placed over the peroneal nerve, just below capitulum fibulae, and the anode just proximal to cathode. The recording surface electrode was placed on the skin over the anterior tibial muscle, where the highest amplitude is measured. The reference electrode was placed over the tendon by the ankle. The ground electrode was placed on the skin between the recording and stimulating electrodes (Figure 1.B).

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A CMAP scan is recorded with QtracS and the TRONDNF protocol. The stimulus intensity is first increased manually to supramaximal levels, and then the program decreases it by 0.2% of the previous stimulation until a response can no longer be recorded from the muscle. The process is identical to the one described in a previous study on the upper extremity (Kristensen et al 2019).

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Examination on the lower limbs requires adjustment to the duration of the stimulus, increasing it to ensure the ability to reach supramaximal stimulation.

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To estimate the number of motor units in the CMAP scan, a model is fitted to the recorded scan. This is accomplished in the MScanFit MUNE part of the QtracP analysis software. This process is described in more detail elsewhere (Bostock, 2016). The analysis provides estimates of the number as well as the sizes of the motor units in the given muscle.

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#### 2.4. Muscle Velocity Recovery Cycles (MVRCs)

MVRCs are performed by stimulating muscle fibers and measuring the response from the same fibers. Stimulation is done with a monopolar needle electrode (25 mm x 26G, TECA), inserted in a muscle away from the neuromuscular endplate. The reference electrode is a surface electrode placed approx. 5 mm from the monopolar needle electrode. Recording is via a concentric needle electrode (25 mm x 30G, Dantec) inserted 20 mm proximal to the stimulating monopolar needle. A ground electrode is placed distal to both needles. The placement of the stimulation and recording electrodes can be seen in Figure 1.C.

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The stimulation is controlled by QtracS running the M3REC3 protocol. From this protocol, two examinations were performed. The first examination uses 0, 1, 2 and 5 conditioning stimuli prior to a test stimulus, with decreasing inter stimulus intervals at 34 steps from 1000ms to 1.4ms. Latencies are recorded from the applied test stimulus to the peaks of the responses from the muscle fibers. The examination is stopped when the latency of the conditioned response becomes longer than that of the unconditioned response. The second examination is the frequency ramp. This examination uses 1 s trains of stimuli, increasing in frequency from 1 to 30 Hz, delivered every 2 s. The measures provided by the analysis are the latencies of the responses to the first and the last stimuli of each train.

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## 2.5 Statistical analysis

Statistical analyses were performed in the QtracP program. Data was normalized by log conversion, where appropriate, and compared between groups with Student's unpaired t-test.

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## 3. Results

### 3.1. Participant demographics

The final 79 patients were separated into two groups based on neuropathy status. Patients with confirmed neuropathy were required to have NCS with two affected nerves from the lower limbs, one of which needed to be the sural and/or distal sural nerve. Forty-seven patients met these requirements and were placed in the group of diabetic patients with NCS-confirmed polyneuropathy (DPN+). The remaining 32 patients were placed in the group of diabetic patients without polyneuropathy (DPN-). Table 1 shows the differences in age and sex between the final three groups. The differences in age were non-significant. This table also shows the NCS results for both sural nerves of the two patient groups, which are one of the most sensitive measures of detecting DPN. Table 2 shows the number of abnormal motor nerve parameters in patients with DPN+ and DPN-. Abnormality was defined by a measurement deviating two standard deviations from the mean of laboratory controls, depending on the measured parameter. Velocities and amplitudes were counted as abnormal if below two standard deviations of the mean, while latency was counted as above. In DPN- group, up to 20.6% of patients had one or more abnormal motor NCS parameter due to the requirement of an abnormal sural and/or distal sural nerve to classify the patient as having polyneuropathy. In DPN+ group, as expected, there was higher incidence of abnormal motor NCS parameters up to 66.7%.

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### 3.2. MScanFit MUNE

Table 3 shows the mean values of the most relevant MUNE parameters for each group. Figure 2 A-D are dot-plots of the four measures for each group. From these results, it is evident that there is a significant reduction in the estimated number of motor units in the anterior tibial muscle (71.3), compared to healthy controls (122.7) ( $p = 3.86E-10$ ). Furthermore, this measure also showed a significant difference between the DPN- group (103.2) and controls ( $p = 0.00328$ ) and between the DPN- and DPN+ patients ( $p = 4.37E-05$ ).

Figure 3 gives an overview of the results of diagnostic accuracy of MScanFit MUNE measures. Fig. 3A shows the ROC curves for discrimination between healthy controls and DPN+ patients, while Fig. 3B shows the discrimination between healthy controls and all the diabetic patients. The areas under the ROC curves in 3A (3B) were 0.900 (0.812) for MScanFit MUNE and similar values for the other MScanFit estimates: 0.885 (0.796) for the largest unit (as % CMAP), 0.889 (0.816) for A50 (the smallest amplitude of the units making up the N50 largest 50% of units), 0.890 (0.815) for N50. These values were all well above those for peak CMAP amplitude, i.e. 0.770 (0.701) for peak CMAP amplitude. To put it another way, for a zero false positive rate, MScanFit MUNE would diagnose 71.7% of DPN+ patients as abnormal and 52.6% of all the patients as abnormal, whereas CMAP amplitude would only diagnose 32.6% and 24.4% respectively as abnormal.

### 3.3.MVRC and Frequency ramp

The most relevant results of the MVRC and Frequency ramp examinations are presented in Table 4 and Figures 4 and 5. When comparing the MVRC parameters, there were no differences between the healthy controls and either patient groups. These include muscle relative refractory period, early supernormality and late supernormality measurements. This is also displayed in Figure 4 where the waveforms of all the groups are very similar and there was no difference between DPN- and DPN+ patient groups. For frequency ramp measurements however, the latency of the first response from trains at both 15 and 30 Hz ( $L_{atf}(15Hz)\%$  and  $L_{atf}(30Hz)\%$ ) differed significantly between the

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control group and both patient groups, but not between the patient groups (Table 4, Figure 5). The peak of the first response at 30 Hz (Pkf(30Hz)%) also differed significantly between the DPN- group and controls, but not between DPN+ group and controls, or between DPN+ and DPN- groups. There is a clear tendency in both Figs. 4 and 5 for the DPN+ and DPN- groups to behave very similarly and to differ from the controls, but none of the differences were prominent.

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#### 4. Discussion

In the present study, the main findings were significantly decreased MScanFit MUNE values in anterior tibial muscle, both in DPN- patients and to a much greater extent in the DPN+ patients.

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However, there were no differences in MVRCs related to neuropathy.

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##### 4.1. MScanFit MUNE shows early motor changes in DPN

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In a prior study, using MScanFit MUNE in the median nerve (Kristensen et al., 2019a), we detected degeneration of the motor fibres in type 2 diabetic patients as early as the sensory, although this

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could not be shown by conventional NCS. We raised the argument that, in contrast to the widely accepted theory, sensory and motor fiber are equally involved in DPN, while motor symptoms or motor NCS findings are masked due to reinnervation. However, that study was performed in an

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upper extremity nerve, which is expected to be affected only in later stages, due to the length-dependent pattern of DPN. We therefore considered it important to test whether similar early motor changes occur in a lower extremity nerve.

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In the present study in anterior tibial muscle, we found a substantial decrease in motor unit numbers in diabetic patients with neuropathy, and ROC analysis confirmed that MScanFit MUNE, with an

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AUC of 0.90, provided a more sensitive test of neuromuscular deficit than CMAP amplitude, with an AUC of 0.77. That this discrepancy was due to collateral innervation was evidenced by a 33%

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increase in mean absolute unit size. More interestingly, there was also a significant loss of motor

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units in the diabetics without neuropathy, although CMAP amplitudes did not reveal a deficit. This does not mean that MUNE was more sensitive than NCS at detecting neuropathy, since the classification as DPN+ required abnormalities in at least two nerves, one being the sural (Dyck et al., 2011). F-wave latency has been proposed as the most sensitive NCS measure for detecting DPN (Andersen et al., 1997), and this is supported by Table 2, which shows the greatest number of abnormalities amongst the DPN- patients as 7, for both peroneal and tibial F-waves. Interestingly this is the same number of patients who had MScanFit MUNE values more than two standard deviations below normal (Fig. 2A) while 9 of the DPN- patients had abnormal A50% values (Fig. 2C). MScanFit MUNE can therefore detect motor deficits in diabetics with comparable sensitivity to the most sensitive sensory NCS measures.

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In the upper extremity, MUNE methods have shown great promise in diagnosis and tracking of progression in ALS (Jacobsen et al., 2017; Jacobsen et al., 2018). In relation to DPN, our previous study seems to be the only one to have applied the MScanFit method to the upper limb of type 2 diabetic patients. Our positive findings encouraged us to perform the present study. MUNE methods in lower extremity have been used to study diabetes in a few studies (Allen et al., 2013; Allen et al., 2014; Toth et al., 2014). Toth and colleagues (Toth et al., 2014) performed incremental stimulation MUNE in 20 type-1 diabetics without clinical signs and symptoms of neuropathy and in 14 healthy subjects, in abductor hallucis and abductor digiti minimi muscles. MUNE values were reduced in both muscles in diabetics while NCS results were normal, suggesting motor axonal loss in diabetics without neuropathy. Although not directly comparable to the present study due to a difference in type of diabetes and a different method of measuring motor unit number, this study does seem to report similar findings to our own. In another study, MUNE derived from decomposition-enhanced quantitative electromyography in anterior tibial muscle showed loss of motor units in diabetics with

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neuropathy compared to healthy controls (Allen et al., 2013). The same group later showed more pronounced changes in anterior tibial muscle than in first dorsal interosseus, consistent with the length-dependent nature of DPN (Allen et al., 2014).

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#### 4.2. Significance of early motor involvement for understanding the pathophysiology of DPN.

Despite extensive studies over many years, and the accumulating evidence for the involvement of many different mechanisms, a recent comprehensive review concluded that it is still uncertain how diabetes targets sensory neurons and whether DPN damage originates in the neuronal cell bodies in the dorsal root ganglia or in the peripheral axons and their associated Schwann cells (Feldman, 2019). These questions depend on the common assumption that diabetic neuropathy preferentially targets sensory axons. Our findings in the current study show, however, that that assumption is untenable, since loss of motor units was clearly demonstrated in patients not classified as neuropathic by sensory NCS. If motor and sensory axons are similarly affected, then it is more likely that the primary targets in DPN are the similar axons in peripheral nerve, rather than the structurally distinct and differently located cell bodies. This is consistent with the fact that the earliest clear sign of metabolic dysfunction in diabetic nerves, their striking resistance to ischaemia, is exhibited by both motor and sensory axons, even in the absence of any neuropathic symptoms (Seneviratne & Peiris, 1968; Weigl et al., 1989). This abnormality, a plausible precursor to DPN, is attributed to increased anaerobic glycolysis, most likely induced by endoneurial hypoxia, since it is also induced by hypoxaemia (Hampton et al., 1989) and can be corrected in experimental animals by oxygen supplementation (Low et al., 1984). Our new findings may help correct the erroneous belief that DPN is primarily sensory, which seems to have distracted attention from the abundant evidence that endoneurial hypoxia has a central role in the pathogenesis of DPN (Nukuda, 2014).

### 4.3. MVRCs in patients with type-2 diabetes

MVRCs have been shown to be sensitive in detecting muscle depolarisation in neurogenic muscles (Witt et al., 2019) and ion channel dysfunction in muscle channelopathies (Tan et al., 2014; Tan et al., 2018). We hypothesised that MVRCs might sensitively show muscle membrane changes in DPN, earlier than NCS, and this could contribute further insights into pathophysiological mechanisms of diabetic neuropathy. The present study is the first to apply MVRC recording technique in patients with diabetes. Interestingly, we found only slight changes in the patients, which were unrelated to whether they had neuropathy or not, despite prominent changes in MScanFit MUNE and changes in CMAP amplitude and NCS. This is in accordance with a former study we performed with MVRCs in ALS (Kristensen et al., 2019b). We could not show any MVRC changes in ALS in anterior tibial muscle, despite substantial denervation. In both ALS and DPN there is substantial collateral reinnervation, which may be sufficient to sustain normal muscle fibre properties. However, in the ALS study we could not exclude the possibility that we may have preferentially selected healthy muscle fibers when adjusting the recording needle to obtain a reliable response. Since the denervation and reinnervation processes are patchy in DPN as in ALS, it is possible that the same explanation is valid for diabetic patients. There were, however, significant differences in the frequency ramp recordings, but the differences were between all diabetics and controls, with no relation to neuropathy. Since these differences were small, it seems fruitless to speculate on their possible origin.

### 4.4. Limitations

This study has some limitations. There were no NCS conducted on controls subjects, which prevented us from directly comparing the ability of MScanFit MUNE to detect DPN with the

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current gold standard of conventional NCS, However, the numbers of DPN- and DPN+ patients with MScanFit measurements outside the limits of controls (Fig. 2) were at least comparable with the numbers with NCS parameters outside our laboratory control limits (Table 2). Another limitation was that MScanFit recordings were in some cases performed with a longer stimulus duration of up to 1 ms, instead of the default 0.2 ms, to be able to reach supramaximal stimulation. However, we do not expect this to have affected our results. In spite of increased duration, we could not reach supramaximal stimulation in some subjects, and these subjects had to be excluded. In some subjects, the MScanFit was too unpleasant in anterior tibial muscle to complete the examination, a limitation which we did not experience in upper extremity nerves. Furthermore, as already stated, we cannot exclude that the negative MVRC results may have been caused by a bias towards recording from healthy muscle fibers.

#### 4.5. Conclusion

We showed in this study that MScanFit method may show sensitively motor unit loss in the anterior tibial muscle in patients with type-2 diabetes, not only in patients with DPN, but also in patients who were not classified as having DPN by NCS. Further research is warranted to examine the utility of MScanFit MUNE as a clinical tool for quantifying motor unit loss in DPN and other neuromuscular disorders. Our evidence that motor fibres are affected as much as sensory fibres in DPN has important implications for understanding the pathophysiology. The near normal MVRC recordings were apparently unaffected by the extensive denervation in DPN+ patients, but the question is raised as to whether the current method is suitable for neuromuscular disorders with a patchy distribution.

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**Table 1. Demographics and NCS results**

	Mean ± SE			P-value
	Controls	DPN-	DPN+	DPN- vs DPN+
Age	61.09 ± 1.45	62.25 ± 1.57	63.64 ± 1.12	p=0.47
Sex (f/m)	14/18	18/14	15/32	
Left sural amp		9.63 ± 0.95	3.52 ± 0.40	1.84E-7
Right sural amp		8.83 ± 0.76	3.78 ± 0.42	4.08E-7
Left sural CV		53.68 ± 0.78	45.29 ± 1.05	1.72E-7
Right sural CV		52.58 ± 0.88	46.76 ± 1.28	0.0058
Peroneal amp		5.96 ± 0.56	3.80 ± 0.53	0.0069
Peroneal CV		45.32 ± 0.73	38.75 ± 0.94	3.22E-6
Tibial amp		16.57 ± 1.05	7.29 ± 0.87	1.84E-8
Tibial CV		46.17 ± 0.69	38.67 ± 1.57	0.0022

Table 2. Number of abnormal NCS parameters for each group			
		DPN-	DPN+
N. Peroneus	CV	3 (8.8%)	23 (47.9%)
	F-Wave	7 (20.6%)	24 (50%)
	DML	4 (11.8%)	11 (22.9%)
N. Tibialis	CMAP AMP	6 (17.6%)	27 (56.3%)
	CV	3 (8.8%)	15 (31.3%)
	F-Wave	7 (20.6%)	32 (66.7%)
	DML	2 (5.9%)	12 (25%)
N. Suralis Dxt	CMAP AMP	2 (5.9%)	19 (39.6%)
	CV	2 (5.9%)	19 (39.6%)
	SNAP	4 (11.8%)	28 (58.3%)

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**Table 3. MScanFit results**

	Mean ( $\pm$ SE)			P-value		
	Controls	DPN-	DPN+	Controls vs DPN-	Controls vs DPN+	DPN- vs DPN+
Peak Amp (mV)	6.85 $\pm$ 0.24	6.33 $\pm$ 0.27	5.14 $\pm$ 0.27	0.159	4.65E-05	0.00353
MScanFit MUNE	122.7 $\pm$ 3.8	103.2 $\pm$ 5.1	71.3 $\pm$ 4.7	0.00328	3.86E-10	4.37E-05
Mean Unit Amp ( $\mu$ V)	55.5 $\times$ $\pm$ 1.03	62.6 $\times$ $\pm$ 1.06	73.7 $\times$ $\pm$ 1.05	0.0488	7.79E-05	0.0339
N50	39.06 $\pm$ 1.49	31.20 $\pm$ 2.05	21.43 $\pm$ 1.64	0.00293	1.22E-09	0.00042
A50 (%)	0.904 $\times$ $\pm$ 1.04	1.17 $\times$ $\pm$ 1.07	1.81 $\times$ $\pm$ 1.08	0.00124	7.30E-09	0.00022
A50 ( $\mu$ V)	60.7 $\times$ $\pm$ 1.04	72.2 $\times$ $\pm$ 1.07	84.9 $\times$ $\pm$ 1.06	0.0253	0.00011	0.0827
Largest unit (%)	2.61 $\times$ $\pm$ 1.04	3.35 $\times$ $\pm$ 1.08	4.73 $\times$ $\pm$ 1.08	0.00808	1.05E-07	0.0027
Largest unit ( $\mu$ V)	182 $\pm$ 9	229 $\pm$ 20	244 $\pm$ 16	0.0332	0.00399	0.56

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**Table 4. MVRC Results**

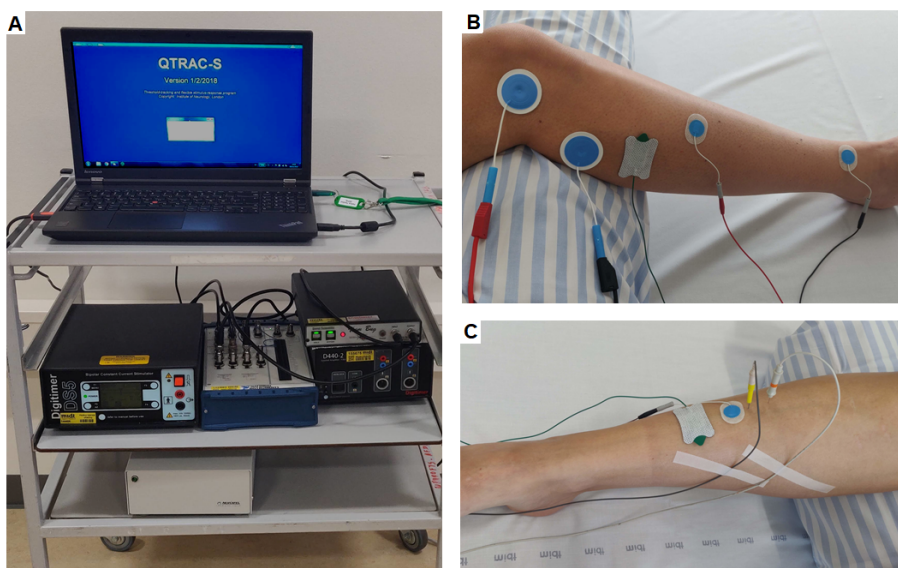
	Mean ( $\pm$ SE)			P-value		
	Controls	DPN-	DPN+	Controls vs DPN-	Controls vs DPN+	DPN- vs DPN+
MRRP	3.31 $\pm$ 0.08	3.50 $\pm$ 0.12	3.48 $\pm$ 0.10	0.205	0.219	0.877
MSuperN(<15)%	12.28 $\pm$ 0.49	11.21 $\pm$ 0.54	12.03 $\pm$ 0.42	0.141	0.703	0.222
M5SuperN(<15)%	15.45 $\pm$ 0.52	14.00 $\pm$ 0.70	14.40 $\pm$ 0.47	0.0955	0.135	0.635
MLSuperN(50-150)%	3.97 $\pm$ 0.20	3.72 $\pm$ 0.28	4.18 $\pm$ 0.16	0.476	0.411	0.105
MXLSuperN(50-150)%	3.13 $\pm$ 0.13	2.92 $\pm$ 0.18	2.95 $\pm$ 0.13	0.336	0.337	0.854
MX5LSuperN(50-150)%	8.64 $\pm$ 0.27	8.14 $\pm$ 0.39	7.99 $\pm$ 0.26	0.289	0.0868	0.739
MRSuperN(950)%	0.14 $\pm$ 0.08	0.12 $\pm$ 0.05	0.25 $\pm$ 0.05	0.804	0.141	0.056
MX5RSuperN(950)%	1.26 $\pm$ 0.09	1.08 $\pm$ 0.13	1.27 $\pm$ 0.11	0.214	0.938	0.247
Peak(mV)	1.62 $\times \pm$ 1.14	1.33 $\times \pm$ 1.17	1.09 $\times \pm$ 1.15	0.346	0.0471	0.187
Lat(15Hz)%	83.2 $\pm$ 0.5	85.6 $\pm$ 0.8	84.7 $\pm$ 0.6	0.0166	0.0672	0.346
Latf(15Hz)%	94.2 $\pm$ 0.5	95.7 $\pm$ 0.4	95.6 $\pm$ 0.4	0.0215	0.0269	0.788
Latf(30Hz)%	94.76 $\pm$ 0.54	96.99 $\pm$ 0.71	96.48 $\pm$ 0.57	0.0144	0.0322	0.588
Pkf(30Hz)%	108.9 $\pm$ 3.9	98.6 $\pm$ 3.1	101.2 $\pm$ 3.6	0.0405	0.149	0.096

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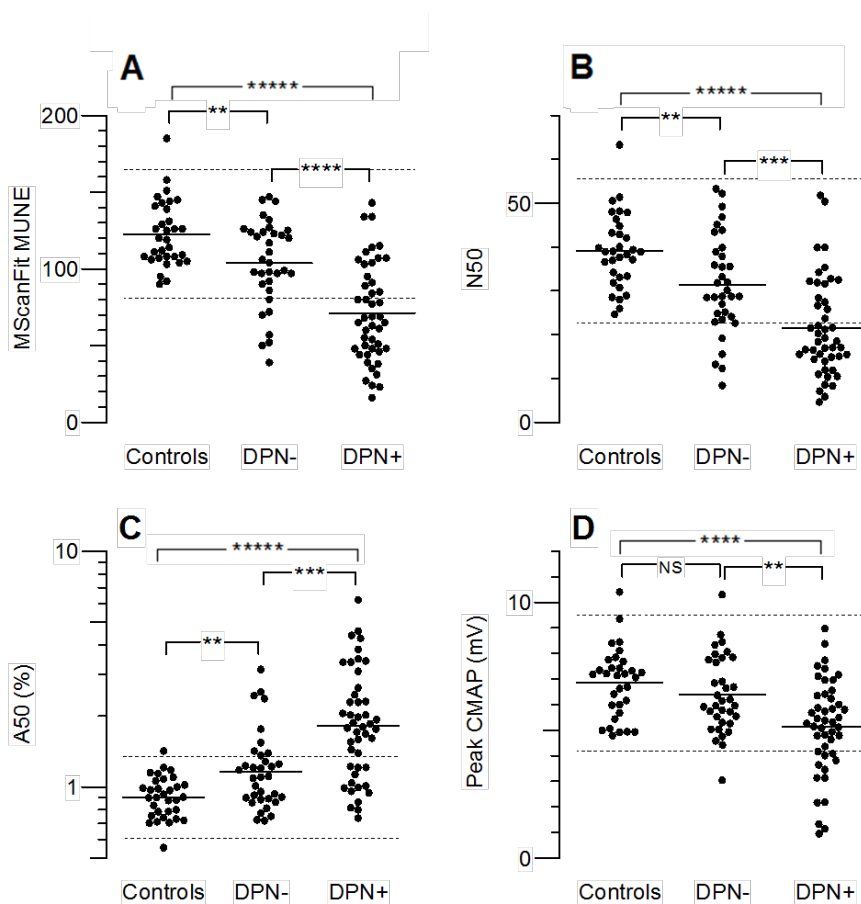


**Figure 1. A:** Hardware for performing MScanFit and MVRCs. **B:** Electrode placement for MScanFit on the anterior tibial muscle. **C:** Needle and electrode placement for performing MVRCs on the anterior tibial muscle.

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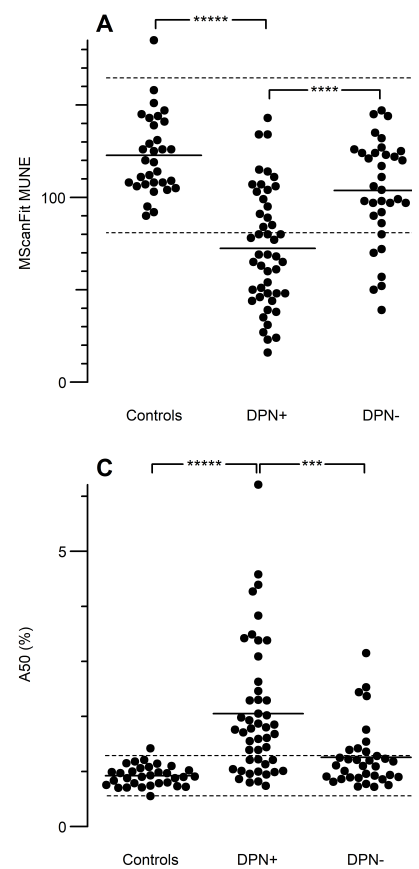


**Figure 2.** Dotplots of the MScanFit measurements with the most significant differences between patient groups and controls. **A:** Number of estimated motor units. **B:** Number of large units that make up 50% of maximal CMAP amplitude. **C:** The smallest motor unit of the units included in N50, relative to maximal CMAP amplitude. (Note logarithmic scale to normalize distributions). **D:** Peak CMAP amplitude. Solid lines are the mean of the group, dashed lines are 95% confidence limits for the control group. Asterisks indicate level of significance (\*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ , \*\*\*\* =  $p < 0.0001$ , \*\*\*\*\* =  $p < 0.00001$ ).



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**Figure 2.** Dotplots of the MScanFit measurements with the most significant differences between patient groups and controls. **A:** Number of estimated motor units. **B:** Number of large units that make up 50% of maximal CMAP amplitude. **C:** The smallest motor unit of the units included in N50, relative to maximal CMAP amplitude. **D:** Peak CMAP amplitude. Solid lines are the mean of the group, dashed lines are 95% CI for the control group. Asterisks indicate level of significance (\* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ , \*\*\*\* =  $p < 0.0001$ , \*\*\*\*\* =  $p < 0.00001$ ).



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Figure 3. ROC curves describing the ability of MScanFit measures and CMAP amplitude to discriminate A: healthy controls from DPN+ patients, and N: healthy controls from all the diabetic patients.

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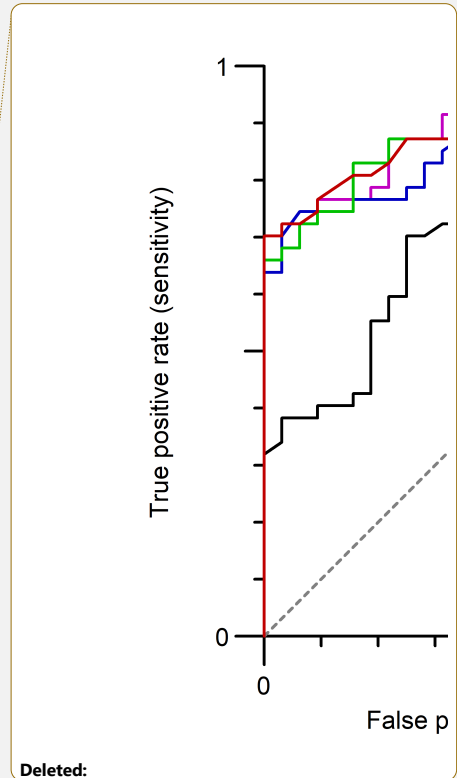
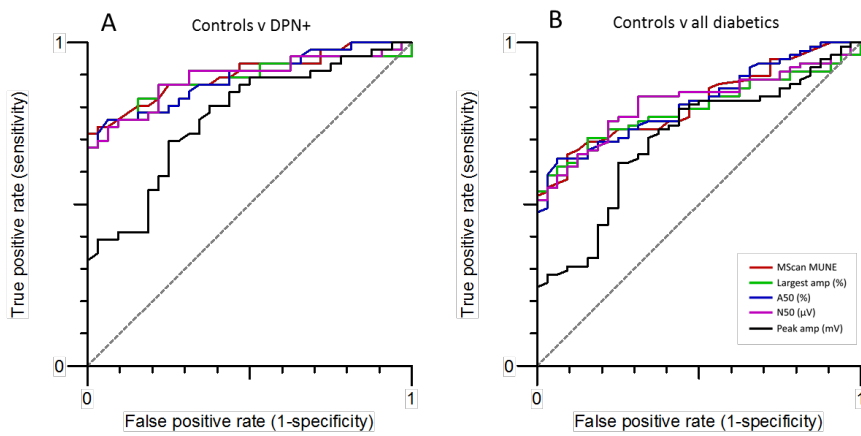
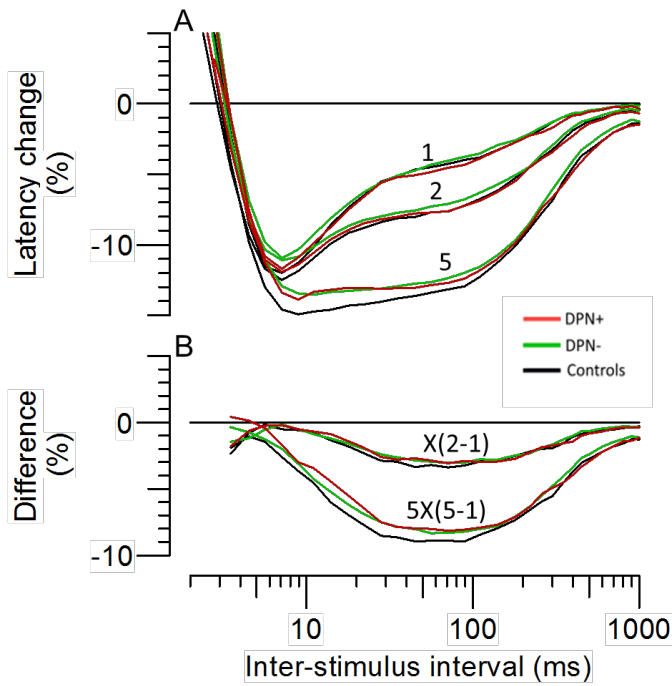


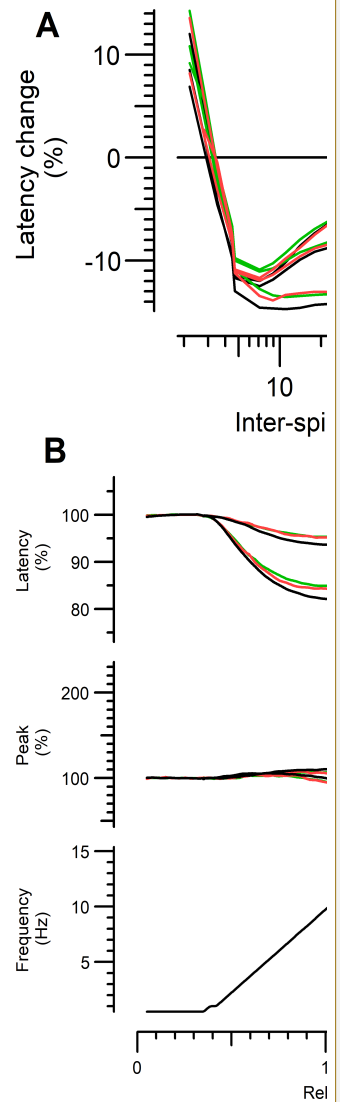
Figure 4. MVRCs

A. Muscle velocity recovery cycles following 1, 2 and 5 conditioning pulses, recorded from healthy controls (black), DPN- patients (green) and DPN+ patients (red). B. Difference plots showing additional latency reductions produced by 2 and 5 compared with 1 conditioning pulse.



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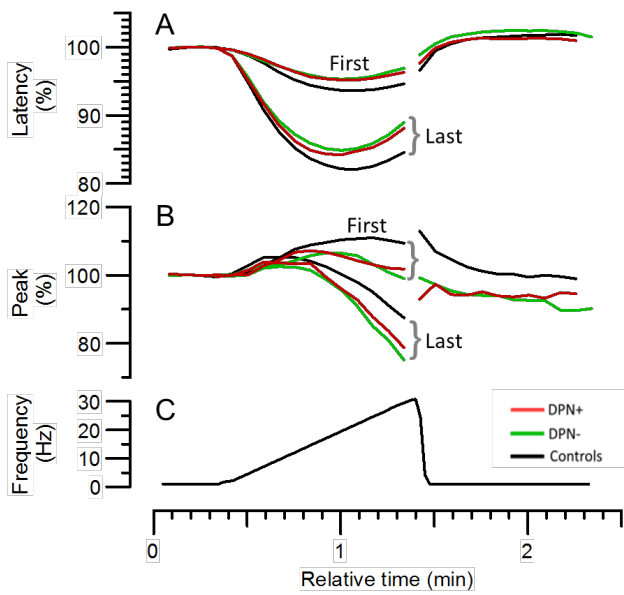
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Figure 5 Frequency ramp responses.

A. Latency changes produced by increasing frequency from 1 to 30 Hz for 1 minute every 2 minutes. Latency changes were recorded to the first and last stimulus in each train, compared with the control value. B. The corresponding changes in peak amplitude. C: Frequency during the trains. Colours as in Fig. 4



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## References

Allen MD, Choi IH, Kimpinski K, Doherty TJ, Rice CL. Motor unit loss and weakness in association with diabetic neuropathy in humans. *Muscle Nerve*. 2013;48:298-300.

Allen MD, Kimpinski K, Doherty TJ, Rice CL. Length dependent loss of motor axons and altered motor unit properties in human diabetic polyneuropathy. *Clin Neurophysiol*. 2014;125:836-43.

Bostock H. Estimating motor unit numbers from a CMAP scan. *Muscle Nerve*. 2016;53:889-96.

Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract*. 2018;138:271-81.

Feldman EL, Callaghan BC, Pop-Busui R, Zochodne DW, Wright DE, Bennett DL, et al. Diabetic neuropathy. *Nature Reviews Disease Primers*. 2019;5.

Feldman EL, Nave KA, Jensen TS, Bennett DLH. New Horizons in Diabetic Neuropathy: Mechanisms, Bioenergetics, and Pain. *Neuron*. 2017;93:1296-313.

Jacobsen AB, Bostock H, Fuglsang-Frederiksen A, Duez L, Beniczky S, Møller AT, et al. Reproducibility, and sensitivity to motor unit loss in amyotrophic lateral sclerosis, of a novel MUNE method: MScanFit MUNE. *Clin Neurophysiol*. 2017;128:1380-8.

Jacobsen AB, Kristensen RS, Witt A, Kristensen AG, Duez L, Beniczky S, et al. The utility of motor unit number estimation methods versus quantitative motor unit potential analysis in diagnosis of ALS. *Clin Neurophysiol*. 2018;129:646-53.

Kristensen AG, Bostock H, Finnerup NB, Andersen H, Jensen TS, Gylfadottir S, et al. Detection of early motor involvement in diabetic polyneuropathy using a novel MUNE method – MScanFit MUNE. *Clin Neurophysiol*. 2019a;130:1981-7.

Kristensen RS, Bostock H, Tan SV, Witt A, Fuglsang-Frederiksen A, Qerama E, et al. MScanFit motor unit number estimation (MScan) and muscle velocity recovery cycle recordings in amyotrophic lateral sclerosis patients. *Clin Neurophysiol*. 2019b;130:1280-8.

Pop-Busui R, Boulton AJM, Feldman EL, Bril V, Freeman R, Malik RA, et al. Diabetic neuropathy: A position statement by the American diabetes association. *Diabetes Care*. 2017;40:136-54.

Tan SV, Z'Graggen WJ, Boërio D, Rayan DR, Norwood F, Ruddy D, et al. Chloride channels in myotonia congenita assessed by velocity recovery cycles. *Muscle Nerve*. 2014;49:845-57.

Tan SV, Z'Graggen WJ, Hanna MG, Bostock H. In vivo assessment of muscle membrane properties in the sodium channel myotonias. *Muscle Nerve*. 2018;57:586-94.

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Toth C, Hebert V, Gougeon C, Virtanen H, Mah JK, Pacaud D. Motor unit number estimations are smaller in children with type 1 diabetes mellitus: A case-cohort study. *Muscle Nerve*. 2014;50:593-8.

Witt A, Kristensen RS, Fuglsang-Frederiksen A, Pedersen TH, Finnerup NB, Kasch H, et al. Muscle velocity recovery cycles in neurogenic muscles. *Clin Neurophysiol*. 2019;130:1520-7.

Z'Graggen WJ, Bostock H. Velocity recovery cycles of human muscle action potentials and their sensitivity to ischemia. *Muscle Nerve*. 2009;39:616-26.

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