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Muscle velocity recovery cycles in myopathy

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HIGHLIGHTS

- Muscle velocity recovery cycles (MVRC), frequency ramp and EMG were performed on 42 patients with myopathy and 42 healthy controls.
- MVRC and frequency ramp measures differed from controls in the non-inflammatory myopathy patients but not in inflammatory myopathy.
- MVRC with frequency ramp may be a useful method for detection of abnormal membrane properties in myopathy of broad aetiology.

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ABSTRACT

Objective: To understand the pathophysiology of myopathies by using muscle velocity recovery cycles (MVRC) and frequency ramp (RAMP) methodologies.
Methods: 42 patients with quantitative electromyography (qEMG) and biopsy or genetic verified myopathy and 42 healthy controls were examined with qEMG, MVRC and RAMP, all recorded from the anterior tibial muscle.
Results: There were significant differences in the motor unit potential (MUP) duration, the early and late supernormalities of the MVRC and the RAMP latencies in myopathy patients compared to controls ($p < 0.05$ apart from muscle relatively refractory period (MRRP)). When dividing into subgroups, the above-mentioned changes in MVRC and RAMP parameters were increased for the patients with non-inflammatory myopathy, while there were no significant changes in the group of patients with inflammatory myopathy.
Conclusions: The MVRC and RAMP parameters can discriminate between healthy controls and myopathy patients, more significantly for non-inflammatory myopathy. MVRC differences with normal MRRP in myopathy differs from other conditions with membrane depolarisation.
Significance: MVCR and RAMP may have a potential in understanding disease pathophysiology in myopathies. The pathogenesis in non-inflammatory myopathy does not seem to be caused by a depolarisation of the resting membrane potential but rather by the change in sodium channels of the muscle membrane.

1. Introduction

Myopathy is caused by a broad group of differing diseases concerning aetiology, characteristics and clinical presentation, but with muscular weakness owing to muscle dysfunction as a common primary symptom (Jackson et al, 2013). Myopathies are often divided into subgroups of non-inflammatory and inflammatory myopathies (Schmidt, 2018), and the diagnostic approach varies considerably at neuromuscular clinics. Thus, the diagnosis of myopathy is based on a wide range of criteria including clinical findings, biochemical tests including muscle enzymes etc., genetic testing or muscle biopsy. Quantitative electromyography (qEMG) strongly increases the diagnostic certainty in many of the

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subgroups as a common denominator and it has been the preferred neurophysiological tool for diagnosis for many years (Fuglsang-Frederiksen, 2006; Liguori et al, 1997; Pugdahl et al 2017). The qEMG recordings sampled at weak effort is the preferred procedure and shows decreased mean amplitude and mean duration of the motor unit action potentials (MUPs) and increased incidence of polyphasic MUPs (Paganoni et al, 2013) in myopathic muscles. Even though qEMG is fast to perform and provides important supplementary information to the clinical findings, it has limitations. It often requires weeks or month of disease until the characteristic qEMG-findings are present, and it is not always sufficient to distinguish between myopathic muscles and an early stage of neurogenic muscle-affection from an EMG recording, since both of these conditions can show polyphasic potentials, fibrillations, positive sharp waves and decreased or increased MUP duration (Liguori et al, 1997). A third pitfall of the qEMG method is the risk of an unconsciously biased selection and editing of the MUPs by the examinator, since the qEMG analysis is still not fully automatic (Fuglsang-Frederiksen, 2006).

The moderate sensitivity of qEMG to myopathy – especially in early stages - and the lack of information on the membrane properties and pathophysiology from this conventional method, leaves a requirement for a supplementary diagnostic tool that can elucidate the membrane properties across the subgroups of myopathies.

In 2009 Z'Graggern and Bostock developed a method called muscle velocity recovery cycles (MVRC) for obtaining information on muscle membrane properties in vivo (Z'Graggen and Bostock, 2009). This work is based on the findings, that the velocity of a second action potential on the muscle fibre membrane changes as a function of the time after the first action potential (Bergmans, 1971; Stalberg 1966). The MVRC method uses direct stimulation of skeletal muscles at rest and makes multifibre recordings that give information on the duration of the refractory period and the depolarizing afterpotential that follows a muscle action potential after conditioning stimuli. These afterpotentials (supernormalities) cause transitory increased excitability and are measured as the reduction of latency of the next muscle action potential following one, two or five conditioning stimuli applied at different interstimulus intervals (ISIs) (Z'Graggen et al, 2009). By recording a MVRC the refractory period and size of the supernormalities can be obtained and provide information of the membrane properties. Since the development of the MVRC, a RAMP protocol has been added to the examination to imitate the short exercise test by exposing the muscle to repetitive stimulations and measuring the effect on the muscle excitability (Boërio et al., 2012).

Earlier studies on the MVRC have shown, that some of the parameters are sensitive to changes in membrane potential (Z'Graggen et al, 2009, Bostock et al. 2012, Humm et al. 2011), and that the measures are abnormal in several neuromuscular diseases such as channelopathies (Tan et al., 2012, Tan et al., 2014, Tan et al., 2016, Boland-Freitas et al., 2018), neurogenic muscles (Witt et al., 2019), critical illness myopathy (Rodriguez et al., 2022, A. Tankisi et al., 2021, Z'Graggen et al., 2011) and in uremic myopathy (Larsen et al., 2021, Z'Graggen et al. 2010). A previous study has shown that MVRC has a high repeatability and is suitable for comparison between both individuals and groups (Z'Graggen et al., 2011, Boërio et al. 2012), but it has only been investigated in a group of healthy subjects, whose muscle membrane properties are expected to be more stable and alike.

To investigate a similar suitability in patients with myopathy and in order to elucidate the membrane changes in this group of neuromuscular disorders, this study examined the MVRC changes in patient with myopathy of broad aetiology, and evaluated the utility of the method as a supplement to the conventional EMG recordings.

2. Materials and methods

2.1. Subjects

The study enrolled 44 patients aged > 18 years and diagnosed with myopathy. Patients were recruited from the Department of Clinical Neurophysiology, the Department of Rheumatology or the Department of Neurology at Aarhus University Hospital (AUH) from 2017-2020, and were contacted by letter or received oral and written information in relation to hospitalization at one of the above-mentioned departments. All patients were diagnosed with qEMG and muscle biopsy, except from a few patients with congenital myopathy who did not have a biopsy but a genetic test.

Symptom duration was listed as months from onset of symptoms until the time of examination. This information was found in the Danish electronic journal system, and since this system only goes ten years back in time, the symptom duration was set to 120 months, in the cases of symptom duration for a longer time than the journal describes.

Additionally, 42 healthy sex- and age matched controls were recruited from the webpage https://www.forsoegsperson.dk or from the staff at AUH and the controls were compared to the patients.

The exclusion criteria for all participants were 1) earlier central or peripheral nervous system disease or nerve damage, 2) known polyneuropathy or conditions that cause polyneuropathy such as diabetes, alcoholism, history of malignancy or medication, 3) use of anticoagulation or any bleeding tendency.

Demographics are listed in Table 1.

The study was approved by the National Committee on Health Research Ethics, and written informed consent was obtained from all participants before participation in the study.

2.2. Preliminary examinations

2.2.1. Neurological examination

All subjects underwent a neurological examination including assessment of muscle strength and trophism, deep tendon reflexes and all sensory modalities (touch, pinprick, vibration, position, temperature) on both upper and lower limbs bilaterally.

2.2.2. Nerve conduction studies (NCS)

NCS were performed on patients and controls in order to make sure, that none of the subject suffered from an undiscovered polyneuropathy or peroneal nerve entrapment neuropathy, and thereby needed to be excluded. Keypoint.Net (Dantec, Skovlunde, Denmark) was used for all recordings following the conventional methods (Stålberg et al., 2019; Tankisi et al., 2019).

Motor NCS were performed on the right n. peroneus with supramaximal stimulus from a handheld bipolar stimulator at following places: 9 cm proximal of the recording electrode at ankle level, distal to capitulum fibulae and at fossa poplitea. The compound muscle action potentials (CMAPs) were recorded from a surface electrode at m. extensor digitorum brevis (EDB) and a reference electrode on the 5th toe. In case of abnormal recordings because of atrophy of the EDB, recordings were performed on m. tibialis anterior, with stimulation of n. peroneus distally of capitulum fibulae and at fossa poplitea.

Sensory NCS was performed on the right n. suralis from a recording-electrode placed between malleolus lateralis and the Achilles tendon, and was stimulated 13 cm proximal from the centre of the recording electrode. Amplitude of the sensory nerve action potentials (SNAPs) were recorded along with conduction velocity.

Table 1
Demographics of patients and controls.

	Controls (n = 42)	Myopathy (n = 42)	*p*-values
Age	52.3 ± 2.33	51.81 ± 2.49	0.858
Sex	22 males 20 females	20 males 22 females	0.775
Type of myopathy vs. controls		**Inflammatory (17):** Polymyositis: 10 Dermatomyositis: 4 Inclusion body myositis: 2 Post-covid myopathy: 1*** Age: 57.88 ± 3.84 (*p*-value: 0.207) Sex: 8 males, 9 females (*p*-value: 0.810) Symptom duration: 73.75 ± 16.05 **Non-inflammatory (25):** Limb Girdle MD: 5 Facioscapulohumeral MD: 7 Duchenne MD: 2 Becker MD: 3 Mitochondrial: 3 Myotonic Dystrophy: 3 Myofibrillar myopathy: 1 Non specified non-inflammatory: 1**** Age: 47.68 ± 3.07 (*p*-value: 0.231) Sex: 12 males, 13 females (*p*-value: 0.889) Symptom duration: 136 ± 15.01	
Inflammatory vs. non-inflammatory		Age: *p* -value: 0.041* Sex: *p* -value: 0.980 Symptom duration: *p* -value: 0.010**	

Age noted as mean ± Standard Error, sex noted as male or female. Symptom duration notes as mean number of months before examination. Compared with a parametric t-test.
MD: Muscular dystrophy.
* p < 0.05, **p < 0.01, *** Inflammatory myopathy has been confirmed with muscle biopsy,
**** Non-inflammatory myopathy has been confirmed with muscle biopsy.

2.3. Quantitative electromyography (qEMG)

qEMG with MUP analysis was performed on m. tibialis anterior using a concentric 37 mm needle electrode (Dantec) and at least 20 MUPs were recorded from 10 different sites at weak voluntary contraction of the muscle.

Duration and amplitude of the MUPs were collected and evaluated in Keypoint. The mean values of the MUPs were calculated and compared, and in case of polyphasia in more than 15 % of the MUPs – interpreted as abnormal -, the mean values of the simple potentials were used for analysis.

Spontaneous activity in the form of fibrillation potentials (fibs) and positive sharp waves (psw) was assessed in the 10 recordings sites, an occurrence of fibs/psw at more than 2 recording sites was regarded as abnormal.

2.4. Muscle velocity recovery cycles (MVRC)

MVRC and the RAMP protocol were carried out by use of the automatic M3REC3 protocol in the QtracS software (developed by H. Bostock, copyright Institute of Neurology, University College London, UK).

Both of the examinations were performed on m. tibialis anterior using a bipolar constant current stimulator (DS5, Digitimer Ltd.) for stimulation through a monopolar 25 mm stimulation needle electrode (TECA elite) placed perpendicularly in the muscle, while a non-polarized surface electrode served as anode just distal of the needle electrode. The muscle activity was recorded from a concentric 25 mm EMG needle electrode (Dantec) placed perpendicular in the muscle 20 mm proximal of the stimulation needle, and the recordings were amplified by an isolated amplifier (D440-02, Digitimer). Noise was eliminated with a HumBug 50/60 Hz noise eliminator (Digitimer, Ltd UK), and the cables were taped to the skin in order to keep them at place during recording.

The stimulation was set to a level of maximum 10 mA with a stable triphasic response, and no change in stimulation current was done during the recording.

The MVRC was recorded after 1,2, and 5 conditioning stimuli separated by 10 ms, and with declining ISIs from the last conditioning stimuli to the test stimuli (1000 ms to 2 ms). Latencies are the time from the test stimulus to the peak of the muscle fiber response.

The RAMP protocol consisted of test stimuli delivered every second. Initially 20 seconds of test stimuli alone was performed, whereupon the test stimu1i continued but were preceded by a 1 second train of conditioning stimuli. The number of stimuli in each train increasing from 1 to 31, and the frequency was raised with 1 Hz for every second cycle. Like that the stimulation rate was ramped from 1 to 15.5 Hz for each minute. Latencies to the responses were measured for trains at 15 and 30 Hz. In the end of the RAMP a 30 seconds period of test stimuli at 0.5 Hz alone was performed.

In order to meet the need of a stable and sufficiently high temperature at the recording site (Bostock et al., 2012), all subjects were heated by use of a heating lamp and the temperature was kept between 32–35 degrees at all recordings of NCS, qEMG and MVRC.

All of the above-mentioned examinations were performed on one occasion.

2.5. Data analysis

2.5.1. Muscle velocity recovery cycles

MVRC latencies were calculated as the time from test stimulus to the highest peak of the following muscle action potential, and the change of the latency after 1, 2 and 5 conditioning stimuli was measured as the percentage difference from the latency for the test stimulus alone without conditioning stimulus.

The following parameters were assessed: 1) Muscle relative refractory period (MRRP) which is the shortest ISI were the latency is the same for the unconditioned and conditioned test stimulus. This parameter is seen as the intersection point with the x-axis of each line in Fig. 1. 2) Early supernormality (ESN) which is the maximum reduction of latency and thereby maximum increase of conduction velocity because of the early afterpotential at < 15 ms ISIs. 3) Time of peak for ESN (ESNt) in ms. 4) Late supernormality (LSN), which is the reduction of latency because of the late afterpotential at 50–150 ms ISIs. Additionally, ESNs were recorded for 5 conditioning stimuli (5ESN) and LSNs were recorded for 2 (XLSN) and 5 (5XLSN) conditioning stimuli.

For the RAMP protocol, latency changes of the negative peak of the muscle action potential was expressed as a percentage change from the baseline latencies and were measured at 15 and 30 Hz of the conditioning stimuli trains. The following parameters were assessed: The latency to the first (Latf(15 Hz)%) and last (Lat (15 Hz) %) response in the train at 15 hz and the latency from the first response in the train at 30 Hz (latf(30 Hz)%).

2.5.2. Statistics

All data from qEMG and MVRC recordings was statistically analysed and figures were generated by the QtracP software developed by Hugh Bostock. Lilliefors test was performed on all data to determine, whether the measures were parametric or non-parametric. An unpaired t-test was used on parametric data, while a Mann-Whitney U-test was used for the non-parametric data. All data is presented as means ± standard error for parametric data or as mean (lower quartile, upper quartile) for non-parametric data. QEMG and MVRC data is presented in tables 2 and 3.

The data from the myopathy patients and the healthy controls were compared to evaluate the suitability of the measures of qEMG and MVRC to discriminate between healthy controls and myopathy patients (Fig. 2, Table 2) and between healthy controls and the two myopathy subgroups respectively (Figs. 2 and 3, Table 2). Results with p < 0.05 were considered significant.

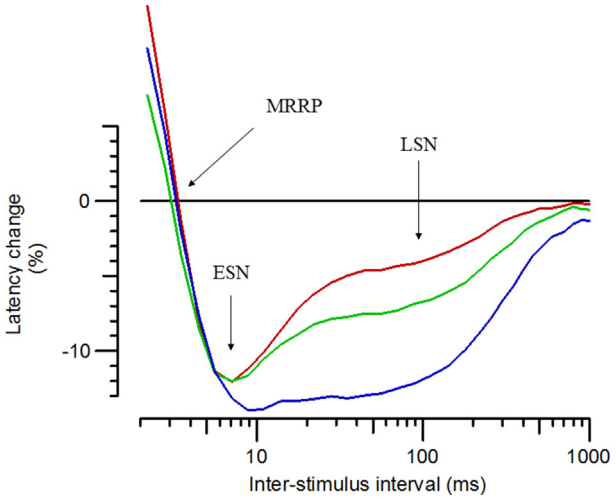

Fig. 1. Muscle velocity recovery cycles in healthy controls depicted as mean. Plotted as the percentage change in latency as a function of interstimulus intervals for 1 (red line), 2 (green line) and 5 (blue line) conditioning stimuli with relative slowing upwards and supernormality downwards. The intersections point with the 0-line, marks the muscle relative refractory period, while the early and late supernormalities are the two wave throughs at respectively 5–15 ms and 50–150 ms interstimulus intervals (ISIs). **MRRP:** Muscle relative refractory period; **LSN:** Late supernormality at 50–150 ms. interstimulus intervals; **ESN:** early supernormality up to 15 ms. interstimulus intervals.

3. Results

3.1. Subjects demographics

One patient was excluded from the study due to the finding of polyneuropathy at the NCS, another patient was excluded due to discomfort at nerve stimulation, while none of the healthy controls were excluded. This left us with 42 controls and 42 patients without a significant difference in gender (healthy controls: 22 males and 20 females and patients: 22 females, 20 males) or age (healthy controls mean age/SE: 52.3 ± 2.33 and patients mean age/SE: 51.81 ± 2.49) between the two groups (Table 1).

Furthermore, the myopathy group was divided into two subgroups as non-inflammatory myopathy (n = 25) and inflammatory myopathy (n = 17) (Table 1). Both of the subgroups were also compared to the control group, and none of the groups differed significantly from the healthy controls concerning age or gender. However, when comparing the two myopathy groups, the non-inflammatory myopathy group was significantly younger than the inflammatory myopathy group, and the period of symptom duration was significantly longer for the non-inflammatory group (Table 1).

One patient with inflammatory myopathy and one healthy control did not participate in the RAMP protocol, respectively because of discomfort and needle movement, and are thereby only a part of the MVRC data. This leaves 41 controls and 41 patients for the RAMP recordings.

3.2. Conventional qEMG findings

The qEMG recordings of the myopathy patients and the controls are compared in Table 2.

The myopathy group had a significantly decreased mean MUP duration compared to the controls (patients mean/SE: 11.48 m s ± 0.28 vs controls mean/SE: 13.57 ms ± 0.19, p=<0.00001) and differed significantly in the mean percentage of MUP-polyphasia.

The control group was compared to the two subgroups of myopathy (Table 3), and a similar tendency was seen as for the entire myopathy group: A significant decrease of mean MUP duration in the group of inflammatory myopathies (mean/SE: 11.45 m s ± 0.4, p=<0.00001) as for the group of non-inflammatory myopathies (11.5 ± 0.39, p=<0.00001) compared to the controls, and a significant difference in the percentage of the polyphasic potentials (Table 3).

Neither the total myopathy group nor any of the subgroups differed significantly from the control group regarding mean MUP amplitude.

For this research we focused on comparing the MUP duration and amplitude of the qEMG to the MVRC measures, and therefore the number of participants with more than two recording sites with spontaneous activity is mentioned in table 2 and 3 without statistical analysis. In 26 % of the patients there were fibs/psw at more than two sites, while this was not found in any of the healthy controls. Regarding the subgroups: 20 % of the non-inflammatory and 35 % of the inflammatory myopathy patients had spontaneous activity.

3.3. MVRC recordings

Comparison of the excitability measures between the controls and the patients appears in Table 2, and the MVRC recordings are illustrated in Fig. 2.

All the specified MVRC measurements apart from the MRRP and ESNt, differed significantly between patients and controls and the two most prominent differences among the MVRC parameters

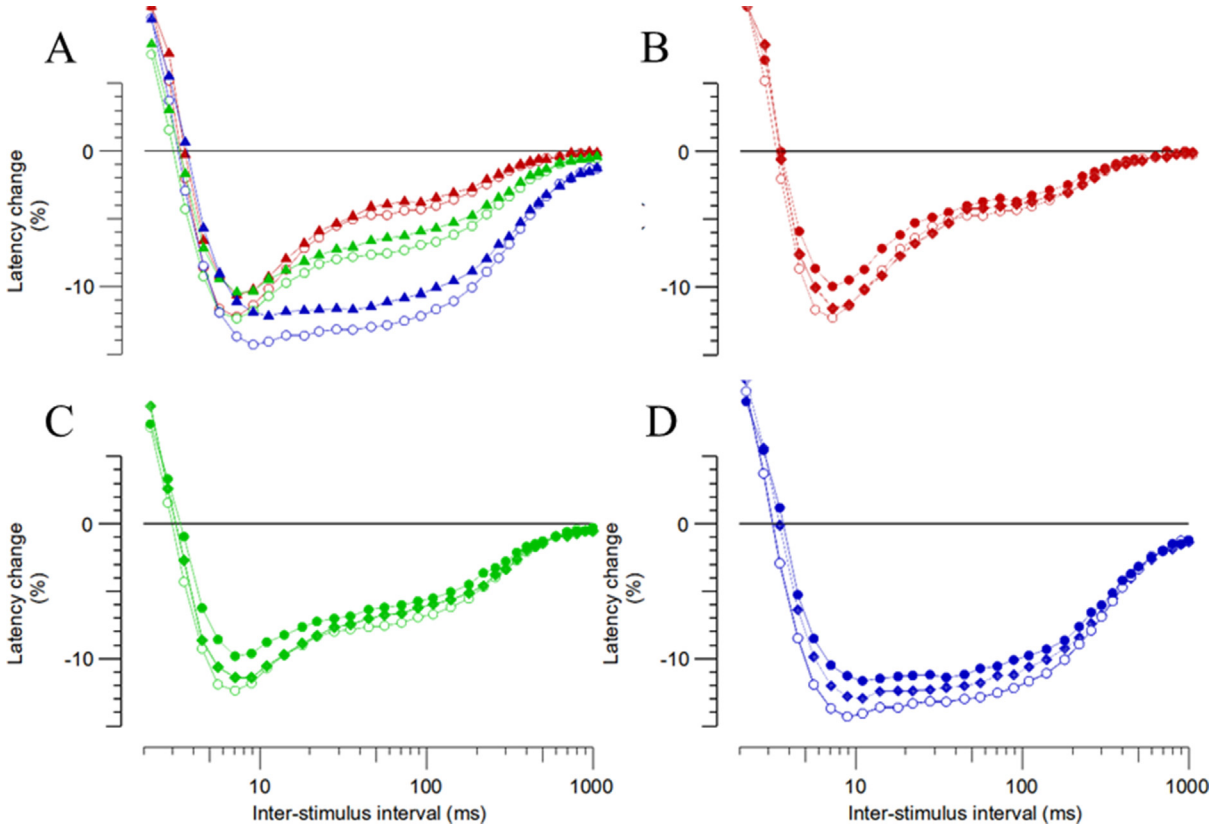

Fig. 2. Muscle velocity recovery cycles (MVRC). Changes are shown as percentage change in latency as a function of interstimulus intervals (ISIs) following 1(red), 2 (green) and 5 (blue) conditioning stimuli. **A:** MVRC in healthy controls (empty circles, n = 42) and all myopathy patients (solid triangles, n = 42). **B:** MVRC with 1 conditioning stimulus in healthy controls (empty circles, n = 42), inflammatory myopathy patients (solid diamonds, n = 17) and non-inflammatory myopathy patients (solid circles, n = 25) **C:** MVRC with 2 conditioning stimuli in healthy controls (empty circles, n = 42), inflammatory myopathy patients (solid diamonds, n = 17) and non-inflammatory myopathy patients (solid circles, n = 25) **D:** MVRC with 5 conditioning stimuli in healthy controls (empty circles, n = 42), inflammatory myopathy patients (solid diamonds, n = 17) and non-inflammatory myopathy patients (solid circles, n = 25).

Table 2
EMG and MVRC measures of controls and patients with myopathy.

Mean ± SE for t-test or Mean (lower quartile, upper quartile) for U-test			
Measure	**Controls**	**Myopathy**	***p*-values**
Fibs/pws(no.subject/all)	0/42	11/42	
MUP duration (ms)	13.57 ± 0.19	11.48 ± 0.28	< 0.00001***
MUP amplitude (μV)	322 (278,408)	310 (238,362)	0.0680
MUP polyphasia(%)	6.65 (4.05, 10.5)	18.2 (9.65,27.5)	< 0.00001***
ESN (%)	12.74 ± 0.47	10.96 ± 0.47	0.00816**
ESNt (ms)	7.1 (5.6, 7.1)	7.1 (7.1, 8.9)	0.29936
5ESN (%)	15.03 ± 0.49	12.77 ± 0.6	0.00433**
LSN (%)	4.26 ± 0.19	3.61 ± 0.26	0.0424*
XLSN (%)	2.73 ± 0.1	2.32 ± 0.12	0.0208*
5XLSN(%)	7.83 ± 0.24	6.86 ± 0.34	0.02033*
MRRP	3.37 ± 0.09	3.55 ± 0.11	0.1905
MLat (15 Hz)%	83.1 (81.6,85)	85.2(83.2,88.1)	0.00839**
Mlatf (15 Hz)%	94.16 ± 0.26	95.89 ± 0.47	0.00194**
Mlatf (30 Hz)%	95.2(93.3, 96.6)	97.2(94.7,98.6)	0.01243*

Fibs/psw: ratio of persons in the group having a mean fibrillation and positive sharp wave ratio > 2/10, **MUP duration:** Mean of the duration of motor unit potential, **MUP amplitude:** The mean of the duration of motor unit potentials, **MUP polyphasia:** the mean of the percentage of MUPs that are polyphasic out of all the MUPS for each person, **ESN:** early supernormality up to 15 ms. interstimulus intervals, **ESNt:** Time to peak of ESN after 1 conditioning stimulus, **5ESN:** early supernormality after 5 conditioning stimuli, **LSN:** Late supernormality at 50–150 ms. interstimulus intervals, **XLSN:** Extra late supernormality after 2 conditioning stimuli, **5XLS:** extra late supernormality after 5 conditioning stimuli, **MRRP:** muscle relative refractory period, **Mlat15Hz:** latency to the last response in the train of 15 Hz in percentage chance from baseline, **Mlatf 15 Hz:** latency to the first response in the train of 15 Hz in percentage chance from baseline, **Mlatf 30 Hz:** latency to the first response in the train of 30 Hz in percentage chance from baseline.
* p < 0.05, **p < 0.01, *** p < 0.001, **SE:** Standard Error.

were the decrease in early supernormality after 1 and 5 conditioning stimuli in the myopathy group compared to the control group. Furthermore, the LSN, XLSN and 5XLSN were also significantly decreased compared to the control group.

As for the RAMP parameters that are illustrated in Fig. 3, there was a significantly smaller reduction in latencies among patients compared to controls, and latf15Hz, lat15Hz and Latf30Hz were all significantly higher for patients.

When dividing into subgroups (Table 3), all of the excitability measures except the MRRP and ESNt were even more significantly different between the non-inflammatory group and the control group, as for the whole myopathy group. On the contrary, none of the excitability measures of the inflammatory group differed significantly from the control group.

The difference in MVRC recordings between controls and the two subgroups are also illustrated in Fig. 2, and the RAMP measures are compared in Fig. 3. Similar comparisons were done as dot-plots in Fig. 4.

There was no significant difference in temperature between the groups prior to or after the electrophysiological examinations.

4. Discussion

In this study we investigated the MVRC parameters as a supplementary diagnostic tool of myopathy and compared it to the conventional qEMG method. In summary, we found that qEMG was able to discriminate between patients and controls based on MUP duration, while the MUP amplitude on the other side does

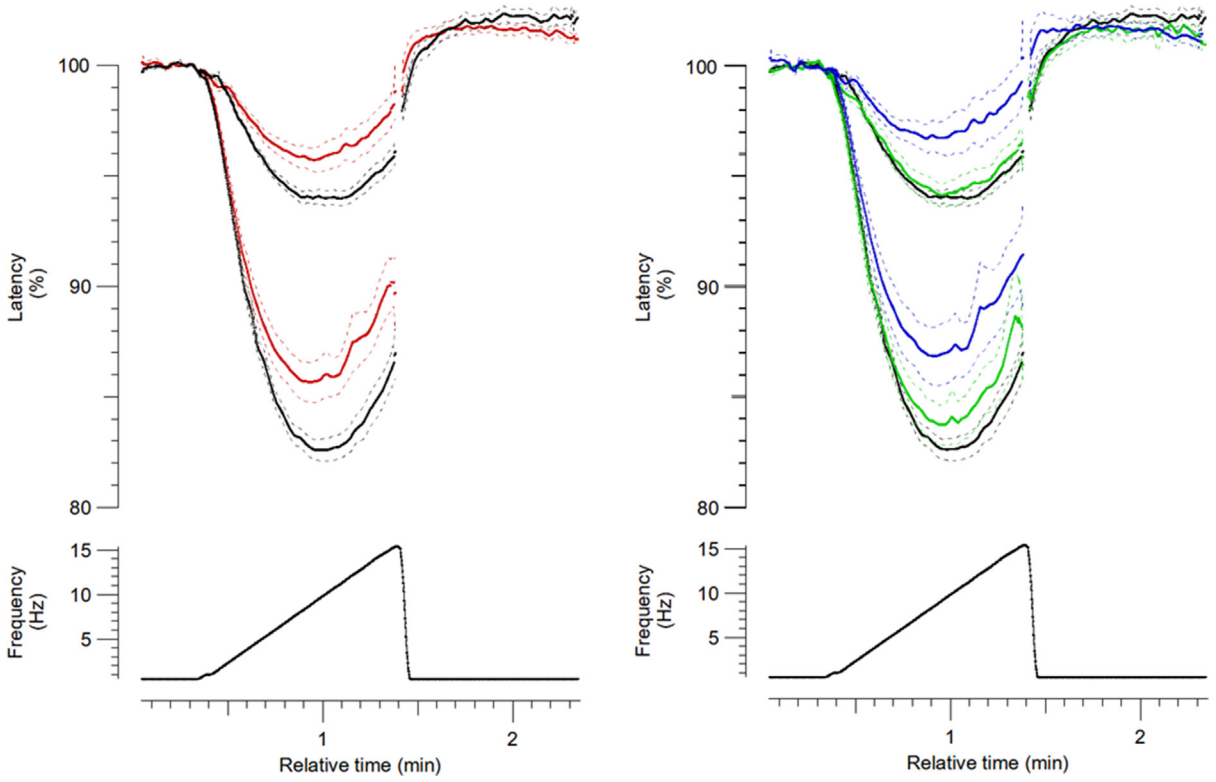

Fig. 3. Frequency ramp for controls (black), all myopathy patients (red), inflammatory myopathy (green) and non-inflammatory myopathy (blue). Mean recordings of percentage change in latency compared to baseline recording for the last (left, lower curve) and the first (left, upper curve) response in trains from 1-30 pr. second delivered every 2nd second. The right curve shows latency changes 30 seconds after end of frequency ramp.

Table 3
EMG and MVRC measures of controls and patients divided into inflammatory myopathy and non-inflammatory myopathy.

Mean ± SE for t-test or Mean (lower quartile, upper quartile) for U-test						
Measure	**Controls**	**Inflammatory**	***p*-values** controls vs. inflammatory	**Non-inflammatory**	***p*-values** controls vs. non-inflammatory	***p*-values** inflammatory vs. non-inflammatory
Fibs/psw(no./all)	0/42	6/17		5/25		
MUP duration (ms)	13.57 ± 0.19	11.45 ± 0.4	< 0.00001***	11.5 ± 0.39	< 0.00001***	0.8888
MUP amplitude (μV)	332 (274, 414)	320 (380, 378)	0.4723	299 (230, 360)	0.0386*	0.1511
MUP polyphasia (%)	6.65 (4.06,10.5)	21.4 (18.2,28.6)	< 0.00001***	15.4 (8.7,18.5)	0.0004***	0.0814
ESN (%)	12.74 ± 0.47	11.8 ± 0.56	0.2540	10.38 ± 0.67	0.0042**	0.1336
ESNt (ms)	7.1 (5.6, 7.1)	7.1 (7.1, 8.9)	0.4601	7.1 (7.1, 8.9)	0.3573	0.9798
5ESN (%)	15.03 ± 0.49	13.29 ± 0.71	0.0516	12.42 ± 0.88	0.0063**	0.4875
LSN (%)	4.14 (3.46,4.95)	3.38 (3.21,4.27)	0.2339	3.44 ± 0.4	0.0378*	0.3470
XLSN(%)	2.73 ± 0.1	2.38 ± 0.21	0.0951	2.28 ± 0.19	0.0257*	0.7290
5XLSN(%)	7.83 ± 0.24	7.13 ± 0.52	0.1635	6.67 ± 0.46	0.0144*	0.5143
MRRP	3.36 (2.93,3.72)	3.47 (3.07, 3.59)	0.5559	3.59 ± 0.14	0.43023	0.5094
MLat (15 Hz)%	83.19 ± 0.42	84.14 ± 0.88	0.2768	86 (85.1,88,4)	0.00141**	0.0952
Mlatf (15 Hz)%	94.16 ± 0.26	94.5 ± 0.523	0.5242	96.78 ± 0.64	0.00007***	0.0153*
Mlatf (30 Hz)%	95.67 ± 0.48	96.05 ± 0.62	0.6613	97.7 (95.4, 98.9)	0.00313**	0.0325*

Fibs/psw: ratio of persons in the group having a mean fibrillation and positive sharp wave ratio > 2/10, **MUP duration:** Mean of the duration of motor unit potential, **MUP amplitude:** The mean of the duration of motor unit potentials, **MUP polyphasia:** the mean of the percentage of MUPs that are polyphasic out of all the MUPS for each person, **ESN:** early supernormality up to 15 ms. interstimulus intervals, **ESNt:** Time to peak of ESN after 1 conditioning stimulus, **5ESN:** early supernormality after 5 conditioning stimuli, **LSN:** Late supernormality at 50–150 ms. interstimulus intervals, **XLSN:** Extra late supernormality after 2 conditioning stimuli, **5XLSN:** extra late supernormality after 5 conditioning stimuli, **MRRP:** muscle relative refractory period, **Mlat 15Hz:** latency to the last response in the train of 15 Hz in percentage chance from baseline, **Mlatf 15 Hz:** latency to the first response in the train of 15 Hz in percentage chance from baseline, **Mlatf 30 Hz:** latency to the first response in the train of 30 Hz in percentage chance from baseline.
* p < 0.05, **p < 0.01, *** p < 0.001, **SE:** Standard Error.

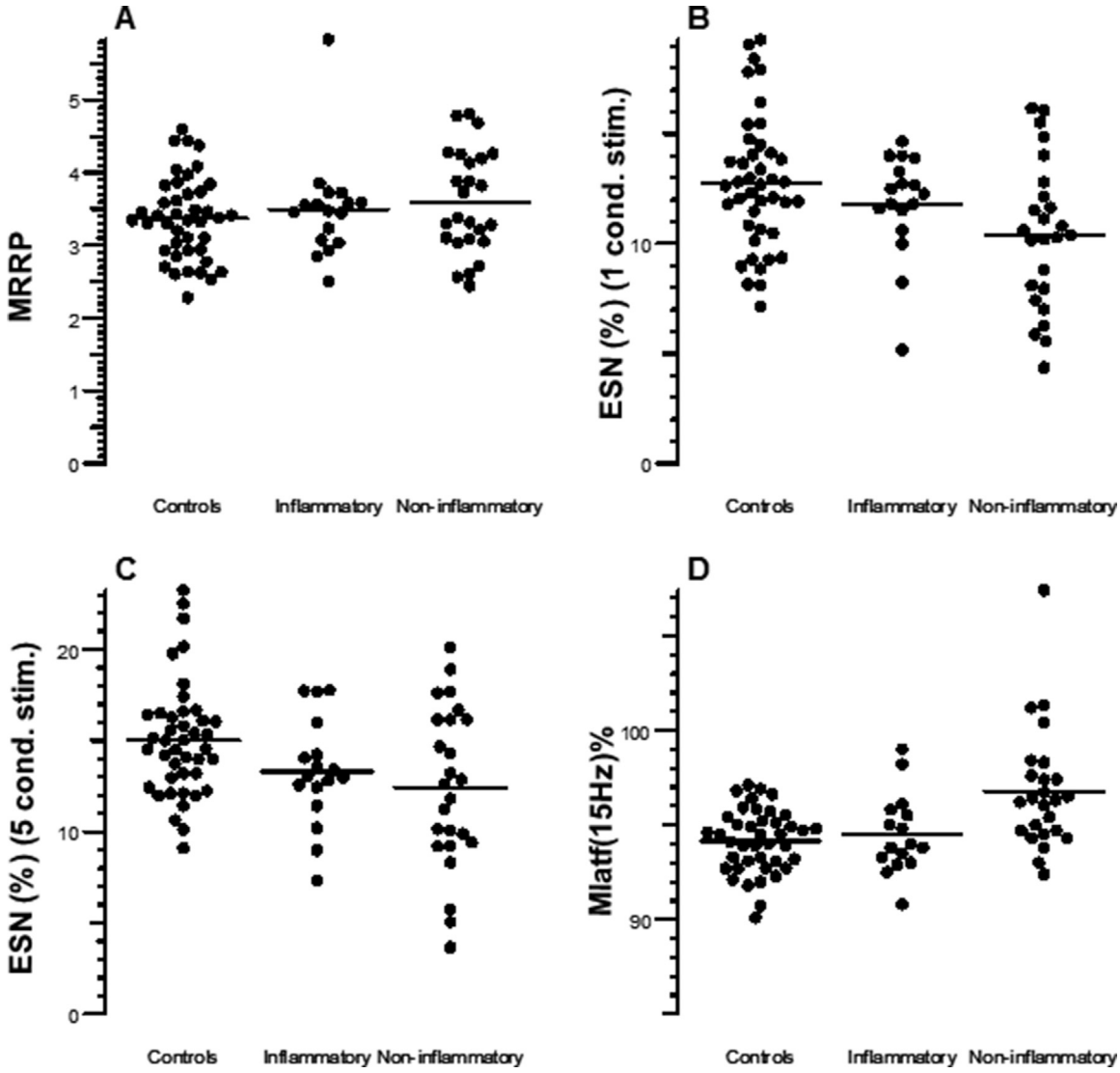

Fig. 4. Muscle velocity recovery cycles (MVRC) and frequency ramp measures presented in dot-plots in healthy controls and inflammatory myopathy and non-inflammatory myopathy patient groups for some selected variables. A) muscle relative refractory period (MRRP), B) Early supernormality, C) 5XLSN: extra late supernormality after 5 conditioning stimuli and D) Mlatf 15 Hz: latency to the first response in the train of 15 Hz in percentage chance from baseline. **ESN:** early supernormality up to 15 ms. interstimulus intervals.

not show any significant changes between the two groups and neither when divided into subgroups of myopathy.

As for the MVRC recordings including RAMP, it was demonstrated that all of the excitability measures except the MRRP and ESNt were significantly different for myopathy patients, and that these differences were only seen in the non-inflammatory myopathy group.

The less prominent increase in the conduction velocity in muscles of the myopathy patients compared to healthy subjects points at a change in the membrane properties of the muscle cells. Since the findings are especially pronounced for the non-inflammatory group, and not significantly present in the inflammatory group, one might think that the mentioned membrane changes are more prominent in the non-inflammatory group than in the inflammatory group.

When comparing to earlier findings, changes in excitability measures have been reported in ischemic muscles (Z'Graggen et al., 2009) and in neurogenic muscles (Witt et al.,2019), and it has been suggested, that the reduction in supernormalities is due to depolarization of the membrane. In these mentioned cases the MRRP was abnormal, which was not seen in the non-inflammatory group of this study. Another study with a similar method has demonstrated that patients with dystrophic muscles have a shorter refractory period, while those of neurogenic muscle have a longer refractory period compared to controls (Mihelin et al., 1991). This confirms that there are differences in the changes of muscle membrane properties and MVRC parameters in different muscle-involving conditions. The decreased supernormalities in combination with the absence of increase in MRRP in the non-inflammatory patients suggests a smaller depolarising afterpotential that is not related to depolarisation of the resting membrane potential in this group. One possible mechanism for the changes of MVRC and RAMP measurements in the non-inflammatory group might be downregulation or reduction of the activity or expression of sodium channels in the muscle membrane leading to a smaller inward sodium current during the action potential. Earlier studies have shown reduced current density of cardiac sodium channels in Duchenne cardiomyocytes (Koenig et al., 2011), allowing us to make this hypothesis of a change in sodium channels in the non-inflammatory myopathy group as a possibility. Further studies to

investigate the channel- and pump function of the muscle membrane are needed to understand the underlying mechanism of non– inflammatory myopathy and its distinction from other membrane-depolarizing conditions, and it might need to include biopsy investigations in comparison with the electrophysiological examinations.

When it comes to our findings in the inflammatory myopathy group, our results are not fully consistent with former studies. As one example, it has been shown, that inclusion body myositis (IBM) involves a significant depolarization of the muscles relatively to healthy controls, which was demonstrated as a reduction in all excitability measures of MVRC including a prolonged MRRP (Lee et al., 2019). Since IBM is an inflammatory myopathy, we expected a similar tendency in this subgroup, but the tendency was more prominent in the non-inflammatory subgroup rather than the inflammatory group. One must mention that the present study only categorized two of the inflammatory myopathy patients as IBM, which makes this group not fully comparable.

There are some possible reasons for the unexpected normal MVRC and RAMP findings in the inflammatory group, and these must be taken into account. One is that this study only investigated the tibialis anterior muscle which might not be the most affected muscle in all of the patients within this group. Most myopathies - for example the group of patients with polymyositis – tend to involve proximal muscles (Schmidt, 2018), while patients with for example Myotonic Dystrophy type 1 are primaly affected in distal muscles and fascial muscles (Johnson, 2019). For some patients with myopathy, the severity and location of muscle weakness also change over time. Despite of these differences in muscle affection, all examinations were done on m. tibialis anterior in order to standardize the examination and measures. This muscle was chosen because the MVRC method is better examined and developed for m. tibialis anterior, and the endplate zone is well defined in this muscle, which is important for the optimal results and the possibility to compare results. If the most affected muscle was chosen, the size of significance levels would likely be bigger for all the methods, including the MUP-duration, because we expect the changes to be biggest in the most affected muscles. Nevertheless, we do see that the qEMG is altered in the muscles of inflammatory myopathy patients, even though these are all collected on the tibialis anterior muscle as well, suggesting that this muscle is in fact affected for inflammatory myopathy patients.

Alternatively, even if the tibialis anterior muscle is involved, the nature of inflammatory myopathy is a patchier muscle involvement, for which reason the MVRC and RAMP recordings might have been made in areas with less pathological fibres. In contrast, qEMG involves sampling from multiple sites which might increase its sensitivity in these conditions. MVRC might therefore be more useful in myopathies with more diffuse involvement of the muscles such as critical illness myopathy (Tankisi et al., 2021) and post-covid myopathy (Agergaard et al., 2021) or multiple recordings should be performed.

It must also be taken into account that most patients with inflammatory myopathy received anti-inflammatory therapy as steroids, which might reduce the severity of abnormal findings.

When comparing the two groups, there are some differences to take into account.

First of all, we found that there was a significant difference in age between the two subgroups (Table 1), and since some of the measures are age-dependent, this is relevant for our findings. However, it has been seen, that higher age generally leads to excitability changes caused by depolarization and thereby reduces ESNs of the MVRC in healthy controls (Lee et al., 2018). Since the non-inflammatory myopathy patients are significantly younger than the inflammatory group, one might suggest that the findings are actually more likely to be an underestimation of a pattern, and it does thereby not reduce the reliability of the results of this subgroup.

Secondly there was difference between the patients regarding time between onset of symptoms or diagnosis of myopathy and the time of our examination. Some patients were examined right after the diagnosis was made, while some patient had the disease for several years before symptoms and diagnosis. It is seen that there is a significant difference in symptom duration between the two subgroups, and this might affect the results of the examinations. It must be taken into account that the symptom duration was not possible to collect with big accuracy, since the Danish hospital records only goes 10 years back. The diagnosis might also not have been added at the actual time of symptom debut, since the process of diagnosis often vary a lot from case to case. It was considered too unreliable to simply ask the patients about their onset of symptoms, since this might be unclear for the patients because of possible insidious development of symptoms. It is still reasonable to assume, that there are differences in the chronic and acute changes in muscle membrane properties, and this can be a part of the explanation of the differences between inflammatory and non-inflammatory group, since the non-inflammatory group might show the chronic changes because of a longer symptom duration.

The difference in spontaneous activity between the two subgroups is probably not connected to the difference in MVRC measures either, since there were fewer patients with spontaneous muscle activity in the non-inflammatory group than in the inflammatory group, and one might expect a high amount of spontaneous activity to be consistent with more severe muscle involvement or depolarisation of the muscle membrane and thereby more pronounced change in excitability measures.

It seems right to continue to see both qEMG recordings and the MVRC recording as a supplement to the clinical, biochemical and genetic test results that makes the myopathy diagnosis today, until more comprehensive studies have elucidated the topic. While MUP duration is reconfirmed as a dependable and sensitive diagnostic tool for myopathy of broad aetiology, it seems like the MVRC is mostly applicable in specific types of myopathy.

5. Limitation

First of all the number of participants could be optimized. With 42 controls and 42 patients, we can definitely deduce overall patterns, but in the light of the interesting findings, it would be preferable to expand the population size. By increasing the number of participants, it would be possible to divide into even more subgroups with a sufficient number of patients, and thereby investigating potential differences of membrane properties among the specific kinds of myopathies within the subgroups. This would enable more focused studies on the future role of the MVRC in elucidating membrane changes and pathophysiology in different types of myopathies. This may be useful in future disease monitoring and contribute to the development of any guidelines for treatment.

Secondly as earlier described, there is heterogeneity in the patient group regarding symptom duration and muscle affection which might blur the results because of its possible affection on membrane properties. We also want to highlight that we did not include neurogenic patients, and we can not be certain about the specificity of the electrophysiological tests including EMG. There are conditions such as Guillain-Barré syndrome that one can see short duration MUPs in early and rapid disease progress.

Having said these things, one of the aims of this study was to test the potential of MVRC in diagnosis of myopathy regardless of affected muscles, period of symptoms or subgroups of myopathy. When we examined all patients the same way regardless of

the above-mentioned differences, we also make bigger demands on the method. If the most affected muscles had been examined, there might have been a bigger significance in the results, but it would not have been appropriate to compare the results of different muscles.

This study elucidates the overall patterns of MVRC in myopathy patients prompting future studies to be conducted in more homogeneous patient population in order to examine different membrane properties between subgroups of myopathy.

In conclusion, several of the MVRC parameters are significantly different between healthy controls and myopathy patients, but this tendency is only evident for the non-inflammatory myopathies, when dividing into subgroups. This might be because of a smaller depolarising afterpotential compared to that of the controls. Since it does not seem to be caused by a depolarisation of the resting membrane, the most probable hypothesis is a change in the sodium channels of the muscle membrane of the non-inflammatory myopathy patients.

MUP duration of qEMG is confirmed to be a very reliable method for diagnosis of myopathy across aetiologies, but MVRC and RAMP recordings may be a supplementary test that reveals different outcome in the subgroups. We suggest that MVRC and frequency ramp in combination with qEMG parameters may help to distinguish inflammatory from non-inflammatory myopathies. However, further studies with more participants within the subgroups are needed to elucidate the precise utility of the MVRC method in diagnosis of myopathy and to fully understand the underlying mechanism of the disease, which seems to be distinct for the different types of myopathies.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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