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Sarcolemmal depolarization in sporadic inclusion body myositis assessed with muscle velocity recovery cycles

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HIGHLIGHTS

- The sarcolemma in sporadic inclusion body myositis is depolarized when compared to normal controls.
- Muscle excitability appears to be differentially affected between muscles in inclusion body myositis.
- Membrane depolarization & weakness may be mediated by amyloid deposition in inclusion body myositis.

ABSTRACT

Objective: To study patients with sporadic inclusion body myositis (sIBM) with muscle velocity recovery cycles (MVRC) to assess muscle membrane excitability, pathophysiological mechanisms and potential biomarkers of this disorder.
Methods: MVRC were recorded from 20 individuals with sIBM from tibialis anterior (TA) and rectus femoris (RF) muscles. Excitability parameters were compared with MVRC data obtained from 22 normal controls >50 years.
Results: Muscle relative refractory period was prolonged in both TA (6.4 ms vs 4.4 ms, $P < 0.001$) and RF (7.1 ms vs 3.9 ms, $P < 0.001$) of sIBM affected muscle when compared to controls. Early supernormality was reduced in both TA (3.6% vs 8.8% $P = 0.001$) and in RF (mean 5.4% vs 13% $P < 0.001$). Late supernormality was only decreased significantly in sIBM affected TA (1.8% vs 3.6% $P = 0.001$) but not in RF. No consistent correlations between MVRC parameters and clinical markers of sIBM disease severity were found.
Conclusion: The resting sarcolemmal muscle membrane potential of sIBM muscle is depolarized relative to that of normal controls, which may be related to intramuscular amyloid deposition in sIBM.
Significance: Sarcolemmal depolarization may play a role in muscle dysfunction and weakness observed in sIBM patients.

1. Introduction

1.1. Sporadic inclusion body myositis

Sporadic inclusion body myositis (sIBM) is the most common acquired myopathy in older adults of the developed world (Needham and Mastaglia, 2016). sIBM causes insidious, relentless progressive muscle wasting over years, selectively affecting muscle groups including the quadriceps and the deep forearm flexors (Needham and Mastaglia, 2016) with sIBM causing significant

Abbreviations: DAP, depolarizing after-potential; EMG, electromyography; ENMC, European Neuromuscular Congress; ESN, early supernormality; IBMFRS, IBM functional rating scale; ISI, inter-stimulus interval; IWCI, IBM weakness composite index; LSN, late supernormality; MFAP, multi-fiber action potential; MRRP, muscle relative refractory period; MVRC, muscle velocity recovery cycle; RF, rectus femoris; sIBM, sporadic inclusion body myopathy; TA, tibialis anterior; 5XLSN, additional late supernormality with 5 conditioning stimuli; XLSN, additional late supernormality with 2 conditioning stimuli.

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morbidity, though not necessarily early mortality in sufferers (Benveniste et al., 2011).

The underlying pathophysiology of sIBM remains poorly understood, with both inflammatory and degenerative mechanisms postulated (Needham and Mastaglia, 2016). The current consensus definition of sIBM is defined by a mixture of clinical, serological and muscle biopsy features. The latest European Neuromuscular Congress (ENMC) criteria (Table 1) proposed assigning patients for research purposes into clinicopathologically defined, clinically defined and probable sIBM classes (Rose et al., 2013). Given its complex definition, sIBM is often misdiagnosed, with variable findings on conventional neurophysiology contributing to a median delay to diagnosis of half a decade (Benveniste et al., 2011).

There is no proven disease modifying treatment for sIBM. The majority of treatment trials to date have employed clinical endpoint criteria such as quadriceps or handgrip strength, functional measures such as the 6-min walk test, or imaging measures of muscle mass over periods ranging frequently between 6 and 12 months (Rose et al., 2015). The difficulty in diagnosis alongside slow progression of sIBM argues the need for a reliable disease marker correlating with clinical function that may assist in future research studies.

1.2. Muscle velocity recovery cycles

The computer-assisted acquisition and measurement of multifiber muscle velocity recovery cycles (MVRC) is a relatively new neurophysiological technique pioneered by Z'Graggen and colleagues (Z'Graggen and Bostock, 2009). MVRC measures the latency and amplitude of a multi-fiber skeletal muscle action potential initiated by a test stimulus, and its variation with the application of previous conditioning stimuli prior to the test stimulus. By varying the number of prior conditioning stimuli and the interstimulus interval (ISI) between the conditioning and test stimuli, a muscle velocity recovery cycle can be constructed. The MVRC thus recorded provides indirect insight into sarcolemmal excitability, resting membrane potential and possibly t-tubule function of the recorded muscle fibers (Z'Graggen and Bostock, 2009).

MVRC is a reliable and reproducible indirect measure of sarcolemmal excitability (Z'Graggen et al., 2011b) and has already been employed in studies of normal muscle physiology, demonstrating the reversible depolarization of the sarcolemmal resting membrane potential (RMP) in response to experimental ischemia (Z'Graggen and Bostock, 2009), hyperpolarization with force training in normal individuals (Z'Graggen et al., 2016b), and relative depolarization with normal ageing (Lee et al., 2018).

In disease states, MVRC has been used to demonstrate sarcolemmal RMP depolarization in renal dysfunction (Z'Graggen et al., 2010) and critical illness myopathy (Z'Graggen et al., 2011a). The impact of myotonic dystrophy on sarcolemmal RMP and chloride conductance in the myotonic dystrophies (Tan et al., 2016; Boland-Freitas et al., 2018), differential membrane properties between varying forms of myotonia congenita (Tan et al., 2014), and differential responses to muscle activation in Andersen Tawil disease (Tan et al., 2012) are other examples of the application of MVRC testing in muscle disease.

1.3. MVRC in sIBM

In this current cross-sectional study, we aim to employ MVRC to interrogate the sarcolemmal potential in sIBM patients. We aim to compare this data with aged normal controls to determine differences in sarcolemmal excitability which may shed light on the pathophysiological mechanisms in sIBM. In addition, correlations of MVRC parameters with clinical disease features will be sought for the suitability of MVRC in tracking sIBM progression.

2. Methods

2.1. Sporadic inclusion body myositis cohort

sIBM patients and normal controls were recruited from the Royal North Shore Hospital Neuromuscular Clinic in Sydney, Australia. Informed consent in accordance with the Declaration of Helsinki was obtained from all patients and the study was approved by the local ethics board.

Twenty sIBM patients were recruited who met the ENMC 2011 consensus sIBM criteria (Table 1) as either clinico-pathologically defined or clinically defined sIBM. A clinical interview and examination were performed on each patient to ensure compliance with the demographic and clinical definitional features of the disease. Each patient had a previous diagnostic muscle biopsy and serum creatine kinase levels measured, with the results of these investigations reviewed to ensure adherence to diagnostic criteria.

2.2. Normal controls

MVRC excitability parameters have been shown to differ between older and younger normal patients (Lee et al., 2018). sIBM is a disease predominantly affecting patients after their 5th decade of life with age > 45yo included in the latest ENMC sIBM definition criteria (Rose et al., 2013).

Therefore, a cohort of normal individuals over the age of 50 years were recruited to serve as controls to provide normative data for MVRC recordings. A clinical interview and physical examination were performed on each control to ensure intact neuromuscular strength globally. Patients with a diagnosed neuromuscular disease, excessive alcohol consumption or diabetes were excluded.

2.3. Clinical markers of sIBM

2.3.1. Clinical markers

The height, weight and calculated body mass index was assessed for each patient. The duration of disease defined from onset of symptoms was determined alongside the age at disease

Table 1
ENMC 2011 Sporadic IBM diagnostic criteria.

		Clinico-pathologically defined IBM	Clinically defined IBM
Demographic	Duration of disease > 12 months	√	√
	Age at onset > 45 years	√	√
Clinical Features	Knee extension weakness ≥ Hip flexion weakness	AND/OR	√
	Finger flexion weakness ≥ shoulder abduction weakness		√
Biochemistry	CK ≤ 15× ULN	√	√
Muscle biopsy features	Endomysial inflammatory infiltrate	√	≥1 of
	Rimmed vacuoles	√	
	Protein accumulation OR 15–18 nm filaments	√	
	MHC Class I upregulation		

onset and diagnosis. The use of immunosuppression has been reported to have a variable but significant effect on sIBM outcomes (Benveniste et al., 2011; Lindberg and Oldfors, 2012). The use of immunosuppressive agents (including IV immunoglobulin, steroids and agents such as methotrexate, azathioprine or mycophenolate) along with the duration of use on history was noted for each subject.

The IBM weakness composite index (IWCI) is a tool scored out of 100, based on clinical assessment of nine neuromuscular power domains that has been described and validated in assessing the clinical severity of sIBM in patients (Benveniste et al., 2011). The IWCI score was calculated for all sIBM participants. Functional impairment of patients with their disease was graded by the previously validated IBM functional rating scale (IBMFRS) (Jackson et al., 2008), scored out of 40 based on patient reports of functioning in ten domains.

2.3.2. Knee extension strength

Quadriceps strength is a key marker of disease in sIBM, with poorer prognostic outcomes for functional independence as strength declines (Allenbach et al., 2012). Quadriceps strength from the limb that the MVRC was recorded from was measured in each sIBM patient. The patient was seated at rest with the knee in flexion at 90 degrees. Peak isometric knee extension strength was recorded, with a dynamometer (SN-500 Dynamometer, Wenzhou Sundoo Instruments) applied just proximal to the ankle malleolus. Three attempts were measured, with the best of these attempts recorded as the peak quadriceps strength, measured in Newtons.

2.4. MVRC recording

MVRC recordings were performed in the tibialis anterior (TA) largely as described by Z'Graggen and colleagues in their seminal work (Z'Graggen and Bostock, 2009) as well as in the rectus femoris (RF) via a similar technique in the right leg of all control and sIBM patients. RF and TA were chosen as candidate muscles in this study as both are clinically affected by sIBM (Needham and Mastaglia, 2016), with the RF portion of the quadriceps being relatively spared in sIBM (Cox et al., 2011).

Bony landmarks were employed to identify each muscle. TA was identified by palpation lateral to the tibial spine, with a stimulating monopolar needle electrode (25 mm × 26G Natus Neurology Incorporated, California USA) inserted into the distal end of the palpated muscle belly, with a surface electrode lateral to the stimulating site acting as the anode. A recording concentric EMG electrode (25 mm × 26G Natus Neurology Incorporated, California USA) was inserted 10–20 mm proximal to the stimulating electrode to record the conducted multi-fiber action potentials (MFAP) and referenced to a surface ground (Fig. 1).

RF was identified in the line between the anterior superior iliac spine and the superior edge of the patella, with the stimulating electrode inserted in the distal 25% of the muscle in an effort to avoid the motor points (Botter et al., 2011). The recording concentric needle was again inserted into the muscle 10–20 mm proximally (Fig. 1).

Square-wave stimuli of 0.01 ms duration were generated via a constant current bipolar stimulator (DS5, Digitimer Ltd, Welwyn Garden City, Hertfordshire, UK). The recorded MFAP from the concentric needle was amplified (1000× gain, bandwidth 1.6–5 kHz) and digitized at a sampling rate of 20kHZ (NIDAQ-6062E, National Instrument Europe Corp., Debrecen, Hungary). Concentric and monopolar needles were adjusted until a stable stimulated MFAP was recorded.

Proprietary QtracS software (Institute of Neurology, London, UK, M3RC3 recording protocol) was employed to track the amplitude and latency of the conducted MFAP peaks generated in response to the stimulus.

Blood was collected within 2 hours of the commencement of recording and serum electrolytes and creatine kinase were assayed for all sIBM patients. Skin surface temperature at a point 2–3 cm lateral to the electrode insertion was recorded at the end of each study.

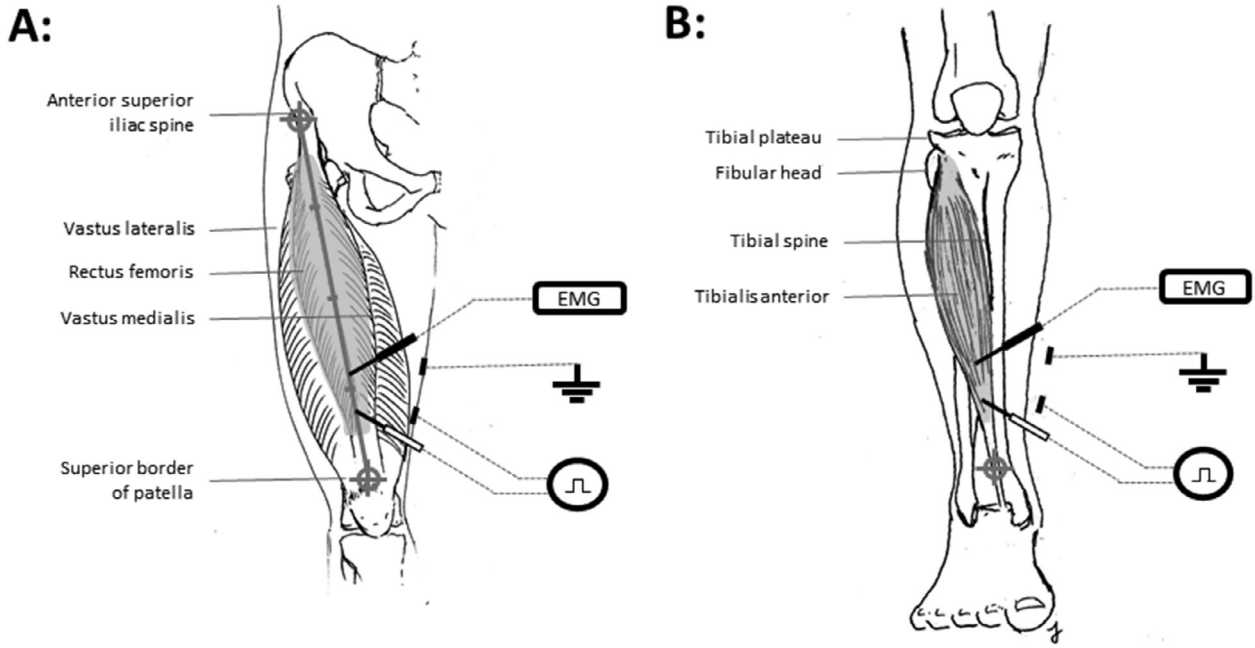

Fig. 1 Experimental MVRC recording setup. (A) Rectus femoris was identified in the line of the anterior superior iliac spine and the patella, with recordings performed around the distal 25% of the muscle to avoid motor points. A monopolar stimulating electrode referenced to a surface anode was applied distally with a recording concentric electrode around 10–20 mm proximal, referenced to a surface ground. (B) Tibialis anterior was identified by palpation lateral to the tibial spine with stimulating and recording electrodes applied at the distal portion of the palpated muscle.

2.5. Recording protocols

2.5.1. MVRC at rest

Resting MVRC was recorded by testing the amplitude and latency to a single unconditioned baseline test stimulus and subsequently comparing this with the MFAP observed after the application of a prior conditioning stimulus. The inter-stimulus interval (ISI) between a single test stimulus and prior conditioning stimuli was varied in a semi-geometric progression between 2 and 1000 ms in steps to build an MVRC curve.

MVRC curves were analyzed to determine excitability parameters as defined by previous authors including muscle relative refractory period (MRRP), early (ESN) and late supernormality (LSN) (Z'Graggen and Bostock, 2009). MRRP was defined as the earliest interpolated ISI at which unconditioned and conditioned test latencies were equal. In recordings where the conditioned MVRC did not cross the unconditioned baseline at ISI out to 1000 ms, no end to the MRRP was identified. These recordings were subsequently excluded from MRRP analysis. ESN was defined as the nadir of the percentage reduction in latency induced by one conditioning stimuli when compared to the unconditioned baseline for ISI < 15 ms. LSN was defined as the average latency reduction induced by one conditioning stimulus when compared to the unconditioned baseline for ISI 50–150 ms. In the event of subjects where conditioned latencies remained prolonged compared to baseline for all ISI recorded, ESN and LSN were correspondingly calculated as negative. These recordings were included as negative latency reduction percentages for ESN and LSN analysis.

Additional conditioning stimuli are known from previous studies to increase the LSN (Z'Graggen and Bostock, 2009). MVRC were recorded with two and five conditioning stimuli instead of one, with the additional LSN thus induced when compared to the single conditioning stimuli defined as the variables XLSN (for two conditioning stimuli) and 5XLSN (for 5 conditioning stimuli) respectively.

2.5.2. Frequency ramp

The second part of the testing involved increasing frequency repetitive stimulation of muscle and recording of the latencies of stimulated MFAPs thus produced, as described by Tan et al. (2014). Briefly, unconditioned baseline test stimuli were recorded for 10 cycles at 0.5 Hz before and through to 30 seconds after repetitive stimulation. The effect of repetitive stimulation was assessed with test stimuli given after a preceding 1 second burst of conditioning stimuli starting at 1 Hz but increasing by 1 Hz every 2 second cycle from 1-30 Hz in a 'frequency ramp'. The response latency at 15 Hz stimulation to the first stimulus in the conditioning train ($Lat(15\ Hz)_{First}$) and to the last test stimulus in train ($Lat(15\ Hz)_{Last}$) were recorded.

2.6. Data analysis

Data analysis was performed using the statistics package on QTracP software. MVRC parameters from normal individuals had relatively low variance compared to the same parameters obtained from sIBM affected patients which displayed far higher variance, and hence Welch's *t*-tests were employed for analysis where both sets of data fulfilled normality tests, with most studied MVRC markers satisfying Lilliefors test for normality at a significance level of 5%.

For those few MVRC parameters that failed Lilliefors test for normality, a non-parametric Welch rank test was performed on the ranks of the data instead to account for non-normality and unequal variance and central tendency of the data was indicated by the median instead of the mean.

Analysis of MRRP, ESN and LSN and their relationship with continuous clinical variables such as duration of disease, age at sIBM onset, quadriceps strength, IWCI, and IBMFRS was performed using simple linear regression after first inspecting the scatterplot for trends and subsequent calculation of attendant *R* and *P*-values, with $P < 0.05$ considered significant.

3. Results

3.1. Inclusion body myositis & normal cohort characteristics

Twenty sIBM patients were recruited, with a mean age at study of 70.8 (±SD 8.0) years. The mean age at sIBM symptom onset was 60.0 (±SD 7.6) years giving a mean disease duration of 10.9 (±SD 6.7) years. Five patients met ENMC 2011 criteria for clinicopathologically defined sIBM, while the remainder met criteria for clinically defined sIBM. This cohort had a median IBM weakness composite index of 60 out of 100 (interquartile range: 49–76), median quadriceps strength of 44.5 N (interquartile range: 30–60 N) and were functionally impaired with a median IBM functional rating scale score of 30 out of 40 (interquartile range: 23–32). Twelve of the twenty patients had received immunosuppressive treatment at some point in their illness. Acceptable MVRC recordings from tibialis anterior were obtained from 19 sIBM subjects and from rectus femoris in 14 sIBM subjects (Table 2).

A cohort of 22 normal controls over the age of 50 years was also recruited with a mean age of 67 (±SD 10.8) years. Twenty-two acceptable TA recordings and seventeen RF recordings were obtained to act as a comparator group.

Serum potassium concentrations (Z'Graggen et al., 2010) and temperature (Bostock et al., 2012) have been described to affect MVRC parameters in disease previously. These variables did not differ significantly between the groups analyzed (Table 3).

3.2. Grouped analysis of sIBM patients versus normal controls

3.2.1. TA recordings

The mean age of the cohort of normal tibialis anterior ($n = 22$) recordings was 67.1 years, similar to the mean age of the 19 sIBM patients with tibialis anterior MVRC recordings at 71.5 years old.

MRRP, ESN and LSN differed significantly between sIBM patients and this >50 year old normal cohort. MRRP was not recordable in two individuals where the conditioned MVRC curve never crossed the unconditioned baseline. In the remaining seventeen sIBM TA recordings, MRRP was significantly longer (median MRRP: 6.4 ms vs 4.4 ms, $P < 0.001$ Welch Rank test). ESN & LSN in TA was negative in three sIBM patients. The mean ESN was significantly reduced when compared to controls (mean ESN: 3.6% vs 8.8%, $P = 0.002$ Welch *T*-test) as was LSN (mean LSN: 1.8% vs 3.6%, $P = 0.003$ Welch *T*-test). Additional LSN with further conditioning stimuli was also reduced when compared to normal with both two conditioning stimuli (mean XLSN: 0.59% vs 2.2%, $P < 0.001$ Welch *T*-test) and five conditioning stimuli (mean 5XLSN: 2.5% vs 5.8%, $P < 0.001$ Welch *T*-test) (Fig. 2, Table 4).

During the frequency ramp part of the protocol, latency compared to baseline was longer in sIBM patients when compared to normal individuals at 15 Hz stimulation both for $Lat(15\ Hz)_{First}$ (99.8% vs 95.5%, $P < 0.001$ Welch *T*-test) and $Lat(15\ Hz)_{Last}$ (91.7% vs 86.9% $P < 0.001$ Welch Rank-test) (Fig. 3).

3.2.2. RF recordings

Fourteen rectus femoris recordings from sIBM affected patients were available for analysis, with an average patient age of 70 years. These were compared with 17 rectus femoris recordings from nor-

Table 2
sIBM cohort characteristics.

Patient Number	Age	Gender	Age at onset + (disease duration)	sIBM Class	IWCI (/ 100)	IBMFRS (/ 40)	Quads strength (N)	IS	TA	RF
1	68	M	63 (5)	C	60	32	31	Y	Y	Y
2	78	F	76 (2)	CP	80	33	58	N	Y	N
3	78	F	58 (20)	C	85	30	41	Y	Y	Y
4	71	M	65 (6)	C	95	37	230	Y	Y	Y
5	85	F	55 (30)	C	45	21	8	Y	Y	N
6	72	M	62 (10)	CP	50	31	9	Y	Y	Y
7	56	F	43 (13)	CP	30	20	36	Y	N	Y
8	70	M	65 (5)	C	60	32	64	Y	Y	N
9	67	M	55 (12)	C	55	26	78	Y	Y	Y
10	61	M	50 (11)	C	60	23	54	Y	Y	Y
11	61	M	55 (6)	CP	55	28	36	Y	Y	Y
12	58	M	54 (4)	C	40	23	33	N	Y	N
13	74	M	68 (6)	CP	75	34	129	N	Y	Y
14	76	F	66 (10)	C	80	35	28	N	Y	Y
15	65	M	55 (10)	C	65	25	51	N	Y	N
16	83	M	71 (12)	C	55	31	48	Y	Y	Y
17	73	M	55 (18)	C	60	30	58	N	Y	Y
18	68	M	60 (8)	C	80	31	230	N	Y	Y
19	73	M	63 (10)	C	30	18	10	N	Y	Y
20	79	F	60 (19)	C	20	18	4	Y	Y	N
	70.8 (MEAN)	14 M 6 F	60 (10.8) (MEAN)	5 = CP 15 = C	60/100 (MEDIAN)	30/40 (MEDIAN)	44.5 N (MEDIAN)	12 IS + 8 IS -	*N* = 19 traces	*N* = 14 traces

Key
CP = clinicopathologically defined sIBM.
C = Clinically defined sIBM.
IS = Previous immunosuppression.
TA – Tibialis anterior MVRC recording present.
RF – Rectus Femoris MVRC recording present.

Table 3
Selected baseline characteristics.

	TA sIBM cohort	TA > 50 yo normal controls	*P*-value	RF sIBM cohort	RF > 50 yo normal controls	*P*-value
Number of individuals	19	22		14	17	
Mean age (years)	72 (±SD 7.4)	67 (±SD 10.8)	$P = 0.1$	70 (±SD 7.2)	67 (±SD 11.5)	$P = 0.4$
Mean serum [Na^+] (mmol/L)	137.5 (±SD 3.8)	139.6 (±SD 2.3)	$P = 0.04$*	138.0 (±SD 3.7)	139.6 (±SD 1.8)	$P = 0.1$
Mean serum [K^+] (mmol/L)	4.6 (±SD 0.4)	4.5 (±SD 0.4)	$P = 0.5$	4.6 (±SD 0.4)	4.5 (±SD 0.5)	$P = 0.3$
Mean skin temperature at recording site (°C)	30.9 (±SD 0.8)	30.6 (±SD 0.7)	$P = 0.3$	30.6 (±SD 1.0)	30.5 (±SD 0.7)	$P = 0.7$

mal individuals over the age of 50 years, with a similar mean age of 67 years.

MRRP was recordable in all fourteen sIBM patients and was significantly prolonged when compared to controls (mean MRRP: 7.1 ms vs 3.9 ms, $P < 0.001$ Welch *T*-test). ESN was negative in one sIBM recording with mean ESN significantly reduced when compared to controls (mean ESN: 5.4% vs 13.0%, $P < 0.001$ Welch *T*-test). Interestingly, unlike in TA, no significant differences were observed in any of the LSN parameters between sIBM patients and normal controls (Fig. 4, Table 4)

In the frequency ramp part of the protocol, sIBM RF recordings demonstrated longer latencies than normal controls for both Lat $(15\ Hz)_{First}$ (99.3% vs 94.0%, $P < 0.001$ Welch *T*-test) and Lat $(15\ Hz)_{Last}$ (92.4% vs 82.9%, $P < 0.001$ Welch *T*-test) (Fig. 3).

3.3. Co-variation of MVRC parameters

MRRP and ESN bore an inverse linear correlation with each other in both the normal and sIBM cohorts in both TA and RF recordings. The inverse linear relationship between MRRP and ESN was seen in TA ($R^2 = 0.59$, $P < 0.0001$), and in RF of the normal cohort ($R^2 = 0.57$, $P = 0.0005$). A significant inverse linear relationship was seen also in sIBM TA recordings ($R^2 = 0.93$, $P < 0.0001$), and sIBM RF recordings ($R^2 = 0.79$, $P < 0.0001$) between MRRP and ESN.

3.4. MVRC parameters and clinical disease severity markers

Correlations were sought in the sIBM patients between muscle excitability markers and clinical markers of sIBM duration and severity of disease.

Excitability markers such as MRRP, ESN and LSN from rectus femoris recordings were analyzed for relationships with key clinical markers in sIBM patients such as duration of disease, IWCI, IBM FRS and quadriceps strength. No statistically significant correlations were found, though the relationship between quadriceps strength and late supernormality with a single conditioning stimulus did approach statistical significance ($P = 0.058$).

Whilst the correlation of LSN with quadriceps strength did not reach statistical significance, the other late supernormality parameters of XLSN and 5XLSN in rectus femoris recordings correlated significantly, albeit weakly with quadriceps strength (Fig. 5). XLSN correlated linearly with quadriceps strength ($R^2 = 0.30$, $P = 0.04$). 5XLSN also correlated linearly with quadriceps strength, ($R^2 = 0.37$, $P = 0.019$).

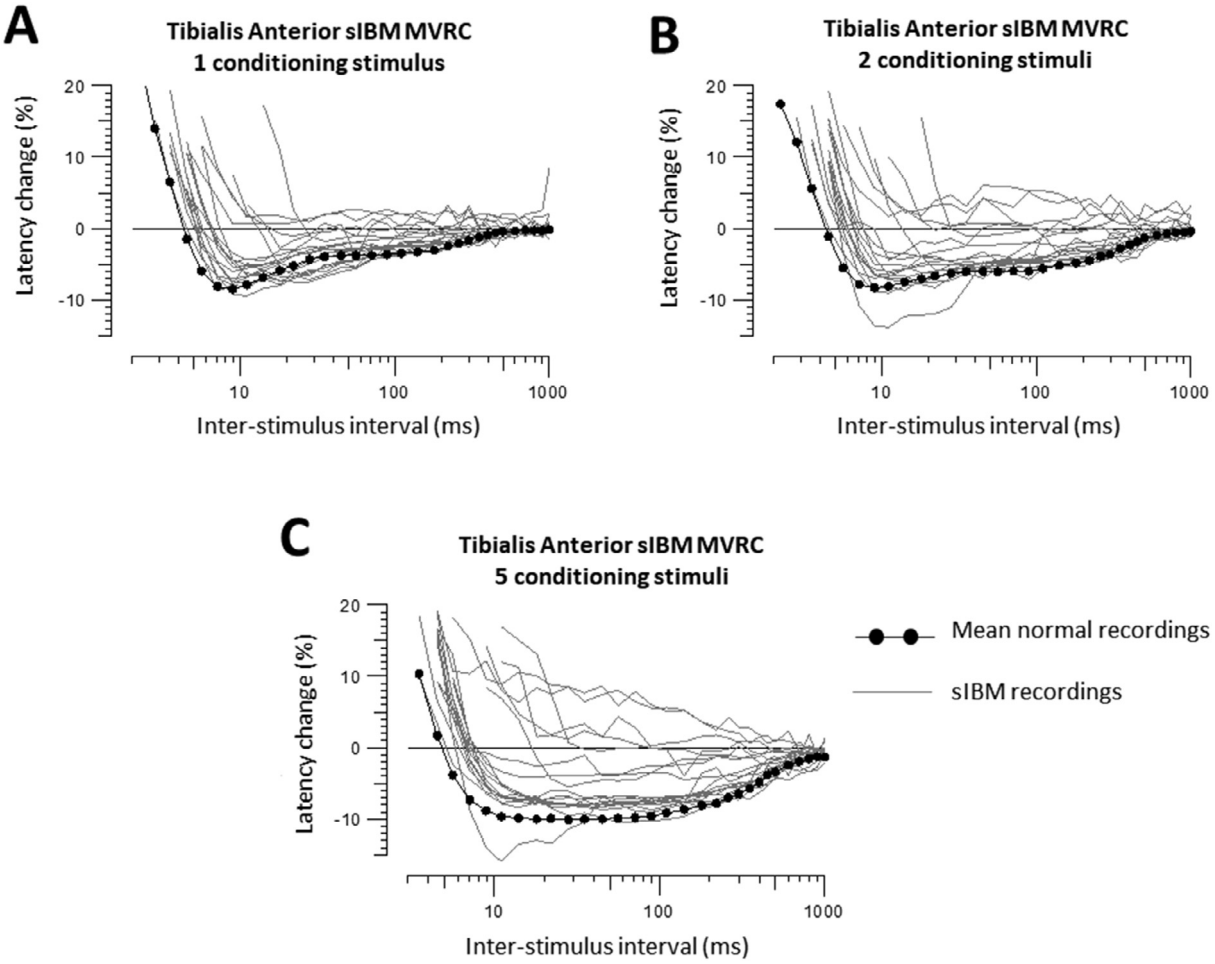

Fig. 2 Tibialis anterior recordings in sIBM patients versus normal controls. MVRC recoordings from tibialis anterior in sIBM patients, with individual sIBM patients in grey, and the mean of normal in black circles. (A) MVRC recordings with 1× conditioning stimulus. (B) MVRC recordings with 2× conditioning stimuli. C: MVRC recordings with 5× conditioning stimuli.

Table 4
MVRC parameters in tibialis anterior and rectus femoris in sIBM muscle versus controls.

	sIBM cohort		Normal cohort		*P*-value
	Mean	Median	Mean	Median	
A: Tibialis Anterior Recordings					
MRRP*	8.5 ms	6.4 ms	4.4 ms	4.4 ms	$P < 0.001$*
ESN	3.6%	5.4%	8.8%	9.0%	$P = 0.001$
LSN	1.8%	2.5%	3.6%	3.2%	$P = 0.003$
XLSN	0.59%	1.2%	2.2%	2.1%	$P < 0.001$
5XLSN	2.5%	3.9%	5.8%	5.6%	$P < 0.001$
$Lat(15\ Hz)_{First}$	99.8%	98.2%	95.5%	95.7%	$P < 0.001$
$Lat(15\ Hz)_{Last}$*	95.8%	91.7%	86.7%	86.9%	$P < 0.001$*
B: Rectus Femoris Recordings					
MRRP	7.1 ms	6.3 ms	3.9 ms	3.9 ms	$P < 0.001$
ESN	5.4%	5.6%	13%	12%	$P < 0.001$
LSN	4.2%	3.9%	5.3%	5.0%	$P = 0.2$
XLSN	2.4%	2.0%	3.4%	3.1%	$P = 0.1$
5XLSN*	5.9%	5.9%	8.5%	7.7%	$P = 0.1$*
$Lat(15\ Hz)_{First}$	99.3%	99.4%	94.0%	94.6%	$P < 0.001$
$Lat(15\ Hz)_{Last}$	92.4%	93.9%	82.9%	84.1%	$P < 0.001$

Notes
* Denotes excitability variables for which data from one of the cohorts did not satisfy normality criteria as determined by Lilliefors test at a significance level of 5%. *P*-values were calculated using a Welch Rank test on these non-normal variables instead of an unequal variance Welch *t*-test which was employed on the remainder of the variables.

No significant relationships were found between excitability markers of MRRP, ESN and LSN and clinical markers of sIBM disease duration, IBM IWCI or IBMFRS in tibialis anterior recordings. A history of immunosuppression has been correlated with poorer outcomes in sIBM. The presence of immunosuppression in the history of our cohort of patients did not correlate with any significant

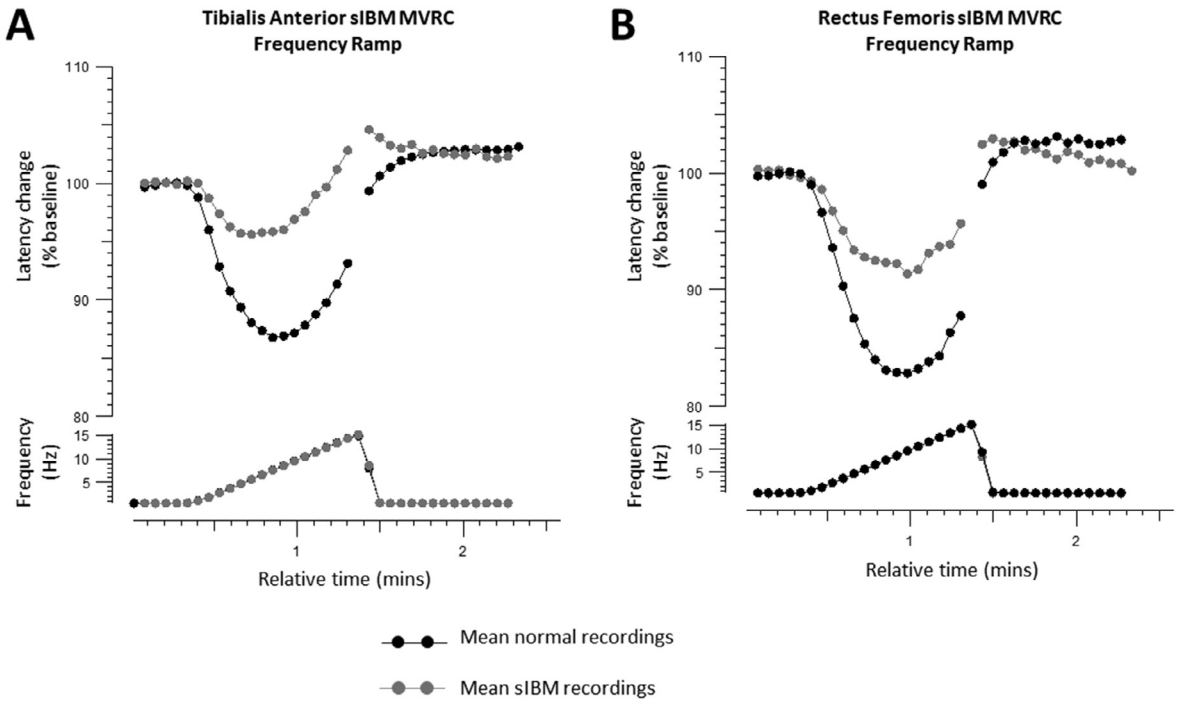

Fig. 3. Frequency Ramp stimulation in sIBM patients versus normal controls. Frequency ramp stimulation data from the last stimulation in the conditioning train with mean of sIBM patients in grey circles, and mean of normal controls in black circles: (A) Frequency ramp recordings from tibialis anterior. (B) Frequency ramp recordings from rectus femoris.

difference in MRRP, ESN or LSN in either tibialis anterior or rectus femoris nor did age at IBM symptom onset.

4. Discussion

The current study employs MVRC in a primarily dystrophic muscle condition with a partially inflammatory etiology. Differences were demonstrated in MRRP, ESN and variably with LSN when compared to older normal controls.

4.1. Relative depolarization of resting sarcolemmal potential in sIBM

The primary finding of this study is that of a prolonged MRRP and reduced ESN in sIBM patients when compared to normal control individuals, in both rectus femoris and tibialis anterior muscles. This pattern of MVRC changes has been reported previously in the muscles of patients with chronic kidney disease (Z'Graggen et al., 2010), critical illness myopathy (Z'Graggen et al., 2011a), and advanced myotonic dystrophy (Boland-Freitas et al., 2018), and has been interpreted in these previous discussions as likely secondary to depolarization of the resting membrane potential by various means. The inverse correlation of MRRP and ESN in both the disease and normal cohorts in this study provides indirect evidence to suggest that both MVRC parameters vary together with another parameter – likely RMP.

The mechanisms tying sarcolemmal depolarization with the MRRP and ESN findings are well described. Depolarization of the resting membrane potential increases membrane refractoriness and decreases muscle excitability via increased inactivation of voltage gated sodium channels, reducing the pool of channels available for the conduction of action potential and thus prolonging the MRRP (Lodish et al., 2000; Filatov et al., 2005). ESN is thought to be mediated by a depolarizing after potential (DAP) following the conduction of an action potential (Z'Graggen and Bostock, 2009), which has been shown in experimental studies to be sensitive to changes in the resting potential with depolarized resting membrane potentials carrying a reduced amplitude, more rapidly decaying DAP in neurons (Barrett and Barrett (1982)). This effect may be mediated by changes in sodium and potassium channel inactivation and transmembrane gradients arising from changes to the resting membrane potential (Z'Graggen et al., 2011a).

Serum potassium levels have a strong effect on MVRC parameters and has been shown to alter MVRC parameters in chronic renal failure patients (Z'Graggen et al., 2010). Potassium does not seem to play a major role in mediating the MVRC changes seen in this study, with serum potassium levels not differing significantly between sIBM and control patients. MRRP and ESN also did not appear to correlate with serum potassium in our sIBM cohort as it does in the setting of chronic kidney disease (Z'Graggen et al., 2010).

Sarcolemmal RMP is largely mediated by the electrogenic Na^+/K^+ ATPase pump and resting open potassium channels (Lodish et al., 2000). Amongst the more interesting of the possible mechanisms through which RMP could be impaired in sIBM involves the direct effect of amyloid beta deposition on muscle function. The deposition of abnormal protein aggregates in muscle has long been described in sIBM muscle (Askanas et al., 2012) and is a key sIBM diagnostic criterion (Rose et al., 2013). One of the most prominent of these abnormal protein aggregations is amyloid beta which has been demonstrated within sIBM muscle (Askanas and Engel (2006), Nogalska et al., 2010) and at increased levels even in the serum of sIBM patients (Abdo et al., 2009). Recent work by Mukhamedyarov has demonstrated that amyloid beta can directly cause membrane depolarization of skeletal muscle (Mukhamedyarov et al., 2009) in a process possibly mediated by direct impairment of Na^+/K^+ pump function (Mukhamedyarov et al., 2014) or indirectly through amyloid mediated mitochondrial

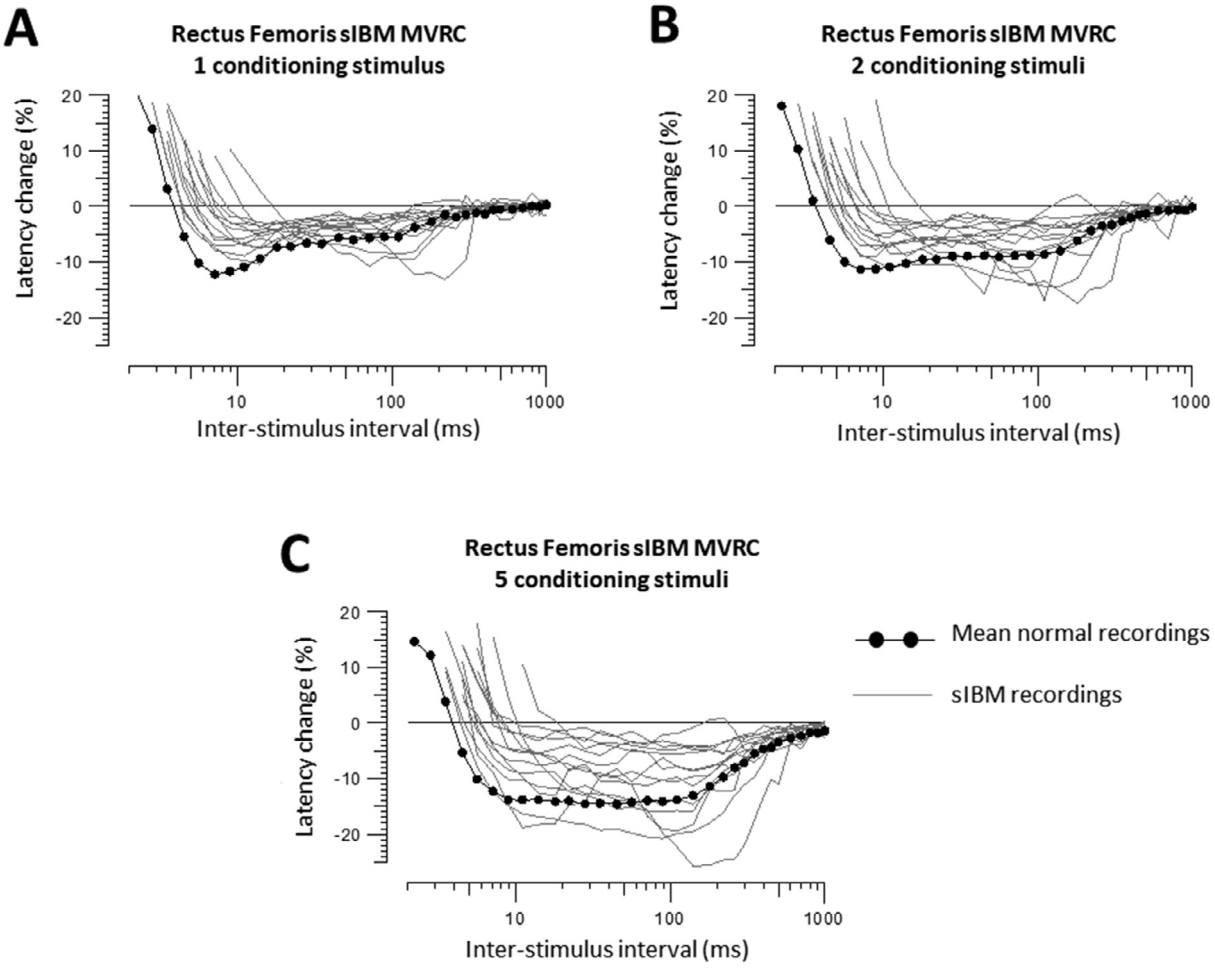

Fig. 4. Rectus femoris recordings in sIBM patients versus normal controls. MVRC recordings from rectus femoris in sIBM patients, with individual sIBM patients in grey, and the mean of normal in black circles. (A) MVRC recordings with 1× conditioning stimulus. (B) MVRC recordings with 2× conditioning stimuli. (C) MVRC recordings with 5× conditioning stimuli.

dysfunction (Boncompagni et al., 2012). Amyloid beta has been shown to impair muscle calcium release and contractility in tissue models (Shtifman et al., 2010; Moussa et al., 2006) providing a mechanism linking sIBM membrane depolarization with muscle weakness.

Though it is more likely that our sIBM MVRC findings are due in major part to a depolarized RMP, the differential possibility of a change in sodium channel inactivation in sIBM explaining the MRRP and ESN findings in this study cannot be entirely discounted, due to well-recognized limitations of this technique (Z'Graggen et al., 2011a). There is some limited prior evidence of differences in sodium channel inactivation in muscle disease with a rat model of critical illness myopathy demonstrating concurrent sodium channel inactivation and resting membrane potential depolarization (Teener and Rich, 2006). We are unaware of any studies on sodium channel activation status in sIBM specifically.

4.2. Late supernormality findings – possible t-tubule dysfunction

We found late supernormality to be significantly reduced in recordings made in sIBM from tibialis anterior, but not rectus femoris, relative to those of normal controls. LSN reflects the degree of potassium accumulation in the t-tubules with the passage of repeated action potentials (Z'Graggen and Bostock, 2009), thus acting as an index of t-tubular function.

Though our findings of reductions in LSN in sIBM may reflect disease related dysfunction of t-tubules, perhaps through inflammatory and degenerative destruction of the t-tubule network, these findings need to be interpreted with caution. ESN and LSN are not mutually independent, ESN having been shown to covary with LSN (Z'Graggen et al., 2011b). The LSN findings in sIBM muscle must thus be cautiously interpreted in the light of the prominent ESN differences, which are of larger magnitude.

It is difficult to account for the differential LSN changes between muscles tested in this study, given the quadriceps complex tends to be affected earlier and more severely in sIBM, though the rectus femoris is preserved until later in the disease (Needham and Mastaglia, 2016). The preferential atrophy and protein loss of Type II fast twitch muscle fibers in sIBM has been described in previous biopsy studies (Arnardottir et al., 2004; Parker et al., 2009). Type II fibers also tend to have a denser t-tubule network than their Type I fiber counterparts (Cullen et al., 1984), and the differential loss of Type II fibers between muscles in sIBM would be a promising explanation for the LSN effects observed in this study but for the fact that the percentage of Type II fibers appears higher in rectus femoris than in tibialis anterior in human autopsy studies (Jennekens et al., 1971; Johnson et al., 1973), a disparity that should argue for a result opposite to that observed in this study.

Whilst the majority of quadriceps weakness in sIBM is almost certainly mediated by significant atrophy of the quadriceps complex, the relationship between LSN and quadriceps strength in

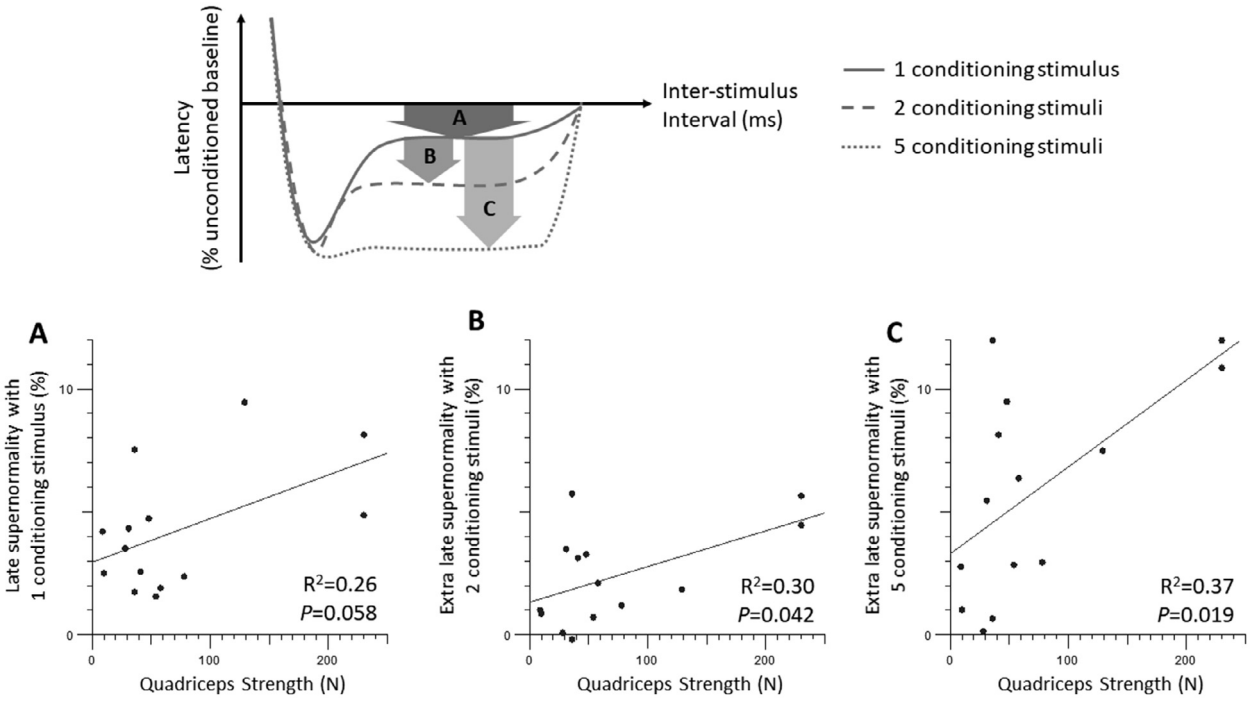

Fig. 5. Correlations of late supernormality with quadriceps strength in rectus femoris of sIBM muscle. In the upper panel, late supernormality induced by a single conditioning stimulus (LSN) is shown with the arrow marked A. The additional late supernormality induced by two and five stimuli respectively (XLSN, 5XLSN) is shown by the arrows marked B and C respectively. The panels below demonstrate the relationships seen between: (A) LSN with a single conditioning stimulus versus quadriceps strength. (B) XLSN with two conditioning stimuli versus quadriceps strength. (C) 5XLSN with five conditioning stimuli versus quadriceps strength.

our sIBM cohort remains of interest. The weak linear relationship between increasing LSN and increasing peak rectus femoris contractile force appeared to strengthen with increasing numbers of conditioning stimuli with the strongest relationship seen with 5XLSN - all of this in the absence of a consistent correlation of quadriceps strength with either MRRP or ESN in the sIBM cohort.

While the dataset is limited with a small number of patients analyzed overall ($n = 14$) and a very limited number of patients with relatively preserved quadriceps strength (>100 N) in our cohort, this finding may tentatively point towards a relationship between LSN and contractile force generation – something that has not as yet been studied in a normal population.

The t-tubules from which LSN is thought to arise are a key link in translating the sarcolemmal action potential into contractile force, carrying the action potential deep into muscle fibers and triggering calcium release from the sarcoplasmic reticulum. Further investigation into correlations between LSN and contractile force in both normal individuals and muscle disease would be of interest.

4.3. Frequency ramp stimulation findings

In both TA and RF recordings, higher frequency stimulation induced less latency reduction from baseline in sIBM patients than controls. The initial latency reduction in the first half of the frequency ramp protocol is thought to be mediated by a similar mechanism as late supernormality through the build-up of potassium in the t-tubules contributing to a depolarizing afterpotential (Boerio et al., 2012). The subsequent prolongation of latencies in the latter half of the frequency ramp is thought to be mediated by progressive sodium channel inactivation in response to membrane depolarization (Boerio et al., 2012; Tan et al., 2016). Our findings in the sIBM cohort of a markedly reduced $Lat(15\ Hz)_{First}$ and $Lat(15\ Hz)_{Last}$ when compared to normal controls is consistent with the postulated depolarized baseline RMP and possible t-tubule dysfunction in sIBM patients that MVRC at rest demonstrated.

4.4. Clinical correlations

Whilst significant on a group analysis when compared against normal individuals, MRRP and ESN did not correlate significantly with most clinical markers of severity including IBMFRS, IWCI or duration of disease within the sIBM cohort. This may reflect our very modest cohort size and also the lack of spread in some of the parameters examined with low numbers of our sIBM cohort having relatively preserved strength or being severely functionally limited by their disease. The utility of MVRC parameters in tracking disease progression within an individual remains unexamined in this study.

4.5. Study limitations

There are several limitations inherent to this study. The first is the intrinsic sampling bias in the needle based multi-fiber MVRC technique. Only relatively excitable, intact muscle fibers from which a reliable MFAP can be generated and recorded would be included in the dataset. This population of reliable fibers may not reflect the broader severity of disease in the muscle sampled. This is supported by the large reported intra-individual variability between MVRC recordings in pathological states such as critical illness myopathy (Z'Graggen et al., 2011a) as opposed to the relative repeatability of MVRC in normal subjects (Z'Graggen et al., 2011b). Muscle fiber involvement in sIBM is patchy and sampling likely to be variable. The employment of a surface electrode technique (Z'Graggen et al., 2016a) for MVRC recording in disease states may allow a more wholistic sampling of an affected muscle which may better correlate with clinical disease severity.

Another weakness is the lack of disease specificity when interpreting our MVRC results. While the changes seen in sIBM are sig-

nificant when compared to normal controls, the prolonged MRRP and reduced ESN is a pattern seen in other disease states such as chronic kidney disease (Z'Graggen et al., 2010) or critical illness myopathy (Z'Graggen et al., 2011a). Such a pattern of presumed depolarization of RMP may be a common neurophysiological picture in neuromuscular disease as a non-specific response to muscle injury – data from an alternative inflammatory muscle disease group would be helpful in exploring this possibility.

5. Conclusion

The current study demonstrates a prolonged MRRP and reduced ESN in sIBM muscle when compared to normal controls, which is favored to represent relative depolarization of the RMP in sIBM muscle fibers. This process is possibly mediated by abnormal amyloid deposition in sIBM muscle. MVRC parameters however did not appear to correlate with other clinical markers of disease severity or duration in this relatively small sample population, with further work required to examine the suitability of MVRC parameters in tracking disease progression over time.

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Declaration of Competing Interest

None of the authors have potential conflicts of interest to be disclosed.

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