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The role of potassium in muscle membrane dysfunction in end-stage renal disease

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HIGHLIGHTS

- Eighteen patients with end-stage renal disease were examined by blood samples and muscle excitability testing before and after hemodialysis.
- Muscle velocity recovery cycles and frequency ramp recordings were strongly associated with potassium levels, showing depolarization before hemodialysis and normalization after.
- Potassium-induced depolarization may be a major cause of muscle fatigue and weakness in end-stage renal disease patients.

ABSTRACT

Objective: Uremic myopathy is a condition seen in end-stage renal disease (ESRD), characterized by muscle weakness and muscle fatigue, in which the pathophysiology is uncertain. The aim of this study was to assess the role of abnormal serum constituents in ESRD patients by relating them to the excitability properties of the tibialis anterior muscle, at rest and during electrically induced muscle activation, by recording muscle velocity recovery cycles (MVRC) and frequency ramp responses.
Methods: Eighteen ESRD patients undergoing hemodialysis were evaluated by blood sample, MVRC, and frequency ramp (before and near the end of dialysis treatment), quantitative electromyography, and nerve conduction studies. Patients were compared to 24 control subjects.
Results: In patients, muscle relative refractory period, early supernormality, late supernormality after 5 conditioning stimuli, and latency of the last of 15 and 30 frequency ramp pulses were strongly associated with potassium levels ($p < 0.01$), showing depolarization before and normalization in the end of hemodialysis.
Conclusions: In ESRD patients, the muscle membrane is depolarized, mainly due to hyperkalemia.
Significance: Since normal muscle fatigue has been attributed to potassium-induced depolarization, it seems likely that this mechanism is also a major cause of the exaggerated muscle fatigue and weakness in ESRD patients.

1. Introduction

Uremic myopathy is a term used in common deficiency of muscle function in patients with end-stage renal disease (ESRD) (Floyd et al., 1974; Serratrice et al., 1967). Uremic myopathy refers to the constellation of functional and occasionally structural muscle abnormalities, in patients with ESRD caused by the uremic state itself (Campistol, 2002). The primary symptom of uremic myopathy is weakness of proximal muscles (Floyd et al., 1974; Quintanilla and Sahgal, 1984), however, the relatively non-specific findings of limited endurance, fatigue, and exercise

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limitation are in most cases the only clinical symptoms (Campistol, 2002).

These non-specific clinical signs are primarily functional in origin, since physical examination, electromyography (EMG), and muscle enzymes are most often normal in ESRD patients diagnosed with uremic myopathy (Floyd et al., 1974), and muscle biopsies only occasionally reveal structural alterations such as muscle fiber atrophy. Most patients are therefore diagnosed clinically (Diesel et al., 1993, Lazaro and Kirshner, 1980).

The exact etiology of uremic myopathy remains uncertain, but it is likely that it has a multifactorial origin, and several interrelated pathogenic mechanisms have been proposed (Al-Hayk and Bertorini, 2007; Campistol, 2002; Kunis et al., 2001; Thompson et al., 1993; Liu et al., 2020; Nishi et al., 2020). Hyperkalemia and acidosis, which are often seen in ESRD patients (Moranne et al., 2009), are two such likely contributing factors, which could account for the functional abnormalities without generating structural changes (Riggs, 1989; Sinha and Agarwal, 2013; Z'Graggen et al., 2010).

By giving ESRD patients 3 hours of 'potassium clamp' dialysis Arnold et al. confirmed that hyperkalemia was responsible for nerve depolarization, and they proposed this chronic depolarization as a cause of uremic neuropathy (Arnold et al., 2014). They also demonstrated a significant increase in walking speed with dietary restriction of potassium in such patients and attributed this to nerve excitability changes (Arnold et al., 2017). It could, however, at least as well be attributed to the (unmeasured) parallel changes in muscle excitability, since hyperkalemia affects muscle and nerve similarly.

In 2009, Z'Graggen and Bostock developed a method to record muscle velocity recovery cycles (MVRC) at rest, offering a clinically attractive approach for the assessment of muscle membrane properties (Z'Graggen and Bostock, 2009). The MVRC method is now an established technique that has been used to determine the excitability properties of skeletal muscle in vivo in several clinical conditions (Kristensen et al., 2020; Kristensen et al., 2019; Lee et al., 2019; Tan et al., 2020; Tankisi et al., 2021; Witt et al., 2020b; Witt et al., 2019). This method uses direct muscle stimulation and multi-fiber recordings to assess the effect of multiple conditioning stimuli on the duration of refractoriness (relative refractory period) and the magnitude and characteristics of periods of transient increased velocity (supernormal periods) following muscle action potentials (Bergmans, 1971; Z'Graggen and Bostock, 2009; Z'Graggen et al., 2011).

In 2010, Z'Graggen et al. conducted a study on MVRCs in ESRD patients undergoing hemodialysis (HD), which revealed chronic depolarization of the muscle membrane mainly caused by hyperkalemia (Z'Graggen et al., 2010). Since then, the original MVRC protocol with 1 and 2 conditioning stimuli has been extended by the addition of an option with 5 conditioning stimuli, and a frequency ramp protocol to detect abnormal responses to repetitive stimulation (Boerio et al., 2012; Tan et al., 2016; Tan et al., 2014; Tan et al., 2018). The frequency ramp protocol imitates the conventional short exercise test by simulating the effect of progressive muscle activation to assess its effect on muscle excitability (Boerio et al., 2012).

This study was undertaken to test if MVRC using additional conditioning stimuli and frequency ramp recordings, and the dependence on serum constituents associated with uremic myopathy on this, could further elucidate the pathophysiology of uremic myopathy in patients with ESRD undergoing HD therapy.

2. Materials and methods

2.1. Subjects

Eighteen patients with ESRD undergoing HD treatment were enrolled in the study. Patients were recruited from the Dialysis Clinic, Aarhus University Hospital (AUH), Denmark from February 2019 to July 2019. Patients were treated by a regimen of HD or HDF (hemodiafiltration) 2–4 times a week, delivered through an arteriovenous fistula (for clinical characteristics see Table 1). Dialysate composition: potassium 2 mM, sodium 138 mM, calcium 1.25 mM, chloride 105 mM, magnesium 0.75 mM, glucose 5 mM, bicarbonate 36 mM, and acetate 3 mM. Subjects were excluded if: they were less than 18 years old, had a medical history of peripheral nervous system disease or entrapment neuropathy, had any coagulopathies, or received anticoagulation therapy (except dalteparin, administered during the HD procedure). Patients suffering from severe physical or cognitive impairment, were also excluded. Furthermore, 24 healthy control subjects similar in age and sex ratio were included. Controls were recruited by advertisements at www.forsoegsperson.dk and AUH concomitantly. The above exclusion criteria also applied to controls and controls were further excluded, if they had one of the following conditions: a medical history of malignancy, diabetes mellitus, alcoholism, medication, or other causes of polyneuropathy (PNP) or myopathy. This study was approved by the Regional Committee on Health Research Ethics (1–10–72–53–17). Written informed consent was obtained from all patients and controls according to the Declaration of Helsinki.

2.2. Examinations

Using same equipment, patients were examined at the Dialysis Clinic, AUH, and controls at the Department of Clinical Neurophysiology, AUH. In patients, clinical evaluation, electrophysiological examination, and blood tests were performed just before HD. Muscle excitability recordings and blood tests were repeated within the last 15 minutes of HD treatment. Controls were examined at one occasion and did not undergo a blood test.

2.2.1. Clinical evaluation

Patients and controls were evaluated clinically by neurological examination including assessment of muscle strength, deep tendon reflexes, and sensory modalities (touch, pinprick, and vibration). Muscle strength in the lower extremities was evaluated using the Medical Research Council (MRC) scale ranging from 0 to 5. Patients were questioned about their subjective experience of generalized muscle fatigue, limited endurance, and exercise limitation after being diagnosed with ESRD.

2.2.2. Laboratory examinations

The blood samples were collected immediately after muscle excitability recordings i.e. right after the patient was connected to the dialysis generator and again within the last minutes of HD treatment. Blood was taken from the arterial needle in the arteriovenous fistula and collected in a blood tube (BD Vacutainer lithium heparin), and a blood gas syringe (Radiometer PICO). ABL835 FLEX was used for blood gas analysis within five minutes after collection of blood and Chemistry XPT for remaining blood analysis. Laboratory examinations included serum levels of potassium, sodium, ionized calcium, phosphorus, chloride, creatinine, carbamide, and blood gas analysis (pH, bicarbonate, and base excess).

2.2.3. Electrophysiological studies

Electrophysiological examinations were performed on the lower leg. The dominant side was chosen for examination. In case of damage to the leg, e.g. scar tissue, the non-dominant side was examined. Skin was cleansed using skin prepping gel and alcohol swipes. The skin temperature of the examined area was retained between 32–35 °C, using a heating lamp when necessary. Skin temperature was measured with an infrared skin thermometer

Table 1
Clinical characteristics of patients with end-stage renal disease.

No.	Sex	Age	Renal diagnosis	Co-morbidities[a]	PNP[b] (±)	PTH[c] (ng/L)	Residual urine output (ml/24 h)	Dialysis vintage (months)	spKt/V [d] (pr session)	UF[e](mL)	Dialysis modality
1	M	39	IgA nephropathy	2	–	912	0	85	1,0	2550	HDF
2	M	48	IgA nephropathy	2	–	887	750	80	1,2	1950	HDF
3	F	49	Unknown	2	–	479	0	124	1,4	3450	HDF
4	F	70	Urolithiasis	4	– [**]	12	0	7	1,3	1500	HD
5	M	52	ADPKD[*]	2	–	250	875	4	1,3	1798	HDF
6	M	44	Hypertensive nephrosclerosis	2	+	258	350	38	1,0	4470	HDF
7	M	72	Hypertensive nephrosclerosis	2	– [**]	486	270	100	1,1	2850	HDF
8	M	48	Hypertensive nephrosclerosis	2	–	924	350	55	1,2	2970	HDF
9	M	63	Hypertensive nephrosclerosis	2	–	48	0	222	1,1	4000	HDF
10	M	55	IgA nephropathy	2	+	195	1500	30	1,1	2200	HDF
11	M	74	Hypertensive nephrosclerosis	2	–	345	1400	29	1,2	1700	HDF
12	M	67	Membranous glomerulonephritis	2	+	10	300	82	1,4	1200	HDF
13	M	79	Mesangial proliferative glomerulonephritis	3	–	101	0	58	1,1	2790	HDF
14	F	70	Glomerulonephritis	2	– [**]	418	1680	89	1,1	200	HDF
15	M	75	Cancer nephrectomy	8	–	190	0	34	1,0	2592	HDF
16	F	71	Proliferative glomerulonephritis	2	–	472	0	35	1,5	2230	HD
17	M	60	Membranous glomerulonephritis	3	+	279	?	2	0,7	200	HDF
18	M	60	ADPKD[*]	2	+	248	627	15	1,3	500	HDF

HDF = hemodiafiltration, HD = hemodialysis.
[a] Charlson comorbidity index (not age-adjusted) based on diagnosis codes from medical journal (Charlson et al., 1987).
[b] PNP = Polyneuropathy based on nerve conduction studies (=abnormality in at least two nerves (Tankisi et al., 2019)).
[c] PTH = Parathyroid hormone levels (measured within 3 weeks before examination).
[d] spKt/V = single pool Kt/V, dialysis dose.
[e] UF = Ultrafiltration.
[*] MPGN = mesangial proliferative glomerulonephritis.
[**] ADPKD = autosomal dominant polycystic kidney disease.
[***] Occurrence of PNP, or not, is based on clinical examination (=impaired ankle reflexes and vibration or pinprick perception (Abraham et al., 2017))

and controlled in the beginning of the examination. Nerve conduction studies (NCS) and EMG were carried out using Keypoint EMG equipment 2.11 (Dantec, Skovlunde, Denmark), while MVRCs and frequency ramp were recorded using a separate set-up as described below.

2.2.3.1. Conventional electrophysiological methods. Routine motor NCS of the peroneal and tibial nerves, and sensory NCS of the sural nerve were performed in all healthy controls and patients to exclude entrapment neuropathy and evaluate the presence of PNP using conventional methods and surface electrodes (Stålberg et al., 2019, Tankisi et al., 2019).

Motor NCS of the peroneal nerve was performed by supramaximal stimulation using a handheld bipolar stimulator at the ankle proximally, and distally to the fibular head. Compound muscle action potentials (CMAPs) were recorded from a surface electrode at the extensor digitorum brevis muscle. Motor NCS of the tibial nerve was performed in the same manner, by stimulating the nerve behind and proximal to the medial malleolus, and in the popliteal fossa. CMAPs were recorded from a surface electrode at the abductor hallucis muscle.

Sensory NCS of the sural nerve was performed by stimulating the nerve 13 cm proximal to the active surface electrode at sura. Sensory nerve action potentials (SNAPs) were recorded from a recording electrode between the lateral malleolus and the Achilles tendon. The evaluated parameters for motor NCS were distal motor latency, conduction velocity, CMAP amplitude, and minimum F-wave latency. For sensory NCS, the conduction velocity and SNAP amplitude were evaluated.

A standard EMG with quantitative motor unit potential (MUP) analysis was performed in the anterior tibial (TA) muscle using a concentric needle electrode for recording (25 mm × 30G, Dantec). Standard filter settings (20 Hz–10 kHz) were used at the department: gain (100 μV/division) and sweep speed (10 ms/division). Quantitative MUP analysis was done by sampling at least 20 different MUPs from 10 different sites in the muscle during weak voluntary contraction. Mean duration, amplitude of all potentials and percentage of polyphasic potentials were evaluated. > 15% polyphasic MUPs are interpreted as abnormal (Stålberg et al., 2019). The presence of spontaneous activity, i.e. fibrillation potentials, positive sharp waves, and fasciculations was also assessed.

2.2.3.2. Muscle excitability recordings. Recordings were performed in the TA muscle in all patients and controls. Stimulation and recording were controlled by the QtracS component of QtracW software (written by H. Bostock, copyright Institute of Neurology, University College London, UK) using the M3REC3 protocol. The recording setup included a DS5 isolated bipolar constant current stimulator, a D440 isolated amplifier, and a HumBug 50 Hz noise eliminator (Digitimer Ltd, UK). Stimulation currents were delivered through an isolated monopolar needle electrode (25mmx26G, TECA), serving as cathode. The needle was inserted perpendicularly into the distal third of the TA muscle. A non-polarizable surface electrode served as anode and was placed on the skin 10 mm distal to the cathode. Muscle recordings were obtained using a concentric needle electrode (25 mm × 30G, Dantec) inserted perpendicularly, 20 mm proximal to the stimulating needle electrode along the muscle fiber direction. A ground electrode was placed distal to

the stimulating needle electrode. The leads were taped to the skin to prevent needle movement during the examination. The needle electrodes were adjusted to obtain a stable single negative peak response with a low stimulation intensity (2.5–7.5 mA). Once set, the stimulation current was not changed during a recording.

MVRCs at rest were recorded following 1, 2, and 5 conditioning stimuli, all separated by 10 ms. The inter-stimulus interval (ISI) between the last conditioning stimulus and the test stimulus varied from 1000 to 1.4 ms in 34 steps in approximately geometric series. Test stimuli were delivered every 2 s. The option to proceed with the frequency ramp follows the MVRCs at rest recording in the M3CR3 protocol. To characterize the effects of progressive muscle activation, excitability measures were derived during repetitive stimulations. In the initial part, 10 cycles of test stimuli alone are delivered at 0.5 Hz. After this period, test stimuli continue to be administered every 2 s. However, they are preceded by 1 s trains of conditioning stimuli that increase linearly in frequency (in 1 Hz steps) to a maximum of 30 Hz over 1 minute. Thereby, over a 1-minute period, the average rate of stimulation is increased from 0.5 to 15.5 Hz. Finally, an additional 30 s period of test stimulation was delivered at 0.5 Hz. The MVRC and frequency ramp stimulation protocols are described elsewhere (Boerio et al., 2012; Z'Graggen et al., 2010) and the examination is presented as a video recording (Witt et al., 2020a).

2.3. Data analysis

2.3.1. Muscle velocity recovery cycles

Latencies were measured from start of the test stimulus to the negative peak of the muscle action potential. The effects of 1, 2, and 5 conditioning pulses on the latency of the test response were calculated as percentage differences compared with the response to the test stimulus alone. Excitability measures derived from MVRC recordings: (1) Muscle relative refractory period (MRRP), defined as the shortest interpolated ISI at which the latencies of the unconditioned and conditioned test response were identical, (2) early supernormality (ESN) measured as the largest percentage latency reduction of conditioned muscle action potential at ISIs < 15 ms, (3) ESN after 5 conditioning stimuli (5ESN), (4) late supernormality (LSN) measured as the average percentage latency reduction between ISIs of 50 and 150 ms, (5) extra-late supernormality following 2 conditioning stimuli (2XLSN) calculated as LSN (2)-LSN, and (6) extra-late supernormality following 5 conditioning stimuli (5XLSN) calculated as LSN(5)-LSN. MRRP and ESN are very sensitive to changes in membrane potential, while LSN is considered to explore changes in transverse tubules (Z'Graggen and Bostock, 2009).

2.3.2. Frequency ramp

Excitability measures made during frequency ramp: The latency of the negative peak of the muscle action potential, expressed as a percentage of baseline latency recorded at 15 Hz (Lat(15 Hz)) and 30 Hz (Lat(30 Hz)) during the ramp and 30 s after the ramp has stopped (Lat(30 Hz + 30 s)). Latency changes were different for the first and last response in each 1 s train of action potentials, and these changes are indicated by the subscripts $_{First}$ and $_{Last}$, respectively, so that Lat(15 Hz)$_{First}$ was the latency to the first of 15 conditioning pulses, expressed as a percentage of the baseline value.

2.3.3. Statistics

Data were exported from QtracP, component of QtracW software, and statistical computations were performed using STATA (16.0, Sweden). QtracP was used to generate figures.

The assumption of normal distribution was checked by plotting QQ-plots and histograms, while SD test (unpaired data) and Bland-Altmann plots (paired data) were used to check the assumption of equal variance.

For parametric data, the student's unpaired t-test with equal variance (5XLSN unequal) was used to compare patients and controls. For non-parametric data, the Wilcoxon rank-sum test was used. Differences between measurements before and after HD were tested using paired sample t-test. Few parameters were log-transformed to obtain normality. If this was not feasible, non-parametric Wilcoxon signed-rank test were performed. Differences are presented as mean difference with 95% confidence interval (95% CI). Log-transformed data were back-transformed and are presented as geometric mean x/÷ SD. Non-parametric comparisons were presented as median difference with interquartile range (IQR). Correlation between changes in excitability measures and serum constituents was assessed using linear regression. Mixed linear regression was applied when looking at all recordings (before and after HD), considering the random effect of subjects. This was relevant since each subject was represented twice in the analysis. When assessing relationships between muscle excitability measures and serum constituents, values of $p < 0.05$ were considered significant.

3. Results

Eighteen patients (14 males and 4 females; mean age: 60.9 years, range 39–79) and 24 controls (16 males and 8 females; mean age: 60.7 years, range 37–75) were enrolled. There was no significant difference in age ($p = 0.91$) and gender ($p = 0.29$) between patients and controls.

3.1. Clinical and conventional NCS and EMG findings

Five patients showed signs of PNP based on NCS and clinical findings (Table 1) while none of the controls had abnormal NCS or clinical findings. Three patients could not tolerate the full extent of NCS because of discomfort arising during supramaximal nerve stimulation. Instead, the occurrence of PNP was evaluated based on clinical findings. No subjects showed signs of peroneal nerve entrapment neuropathy according to criteria developed by Danish Consensus Group (Fuglsang-Frederiksen and Pugdahl, 2011). All subjects showed normal muscle strength (MRC = 5) in lower limbs and hip girdle at the clinical evaluation. However, all patients described a feeling of increased fatigue, limited endurance, and exercise limitation arising after being diagnosed with ESRD. EMG was only available in 17 patients and 18 controls, due to late implementation of EMG on the control subjects (6 controls) and discomfort arising during examination (1 patient). The patient group did not differ significantly from controls with regards to MUP duration (patients mean = 14.91 ms (95% CI 14.04; 15.78) vs controls mean = 14.01 ms (95% CI 13.59; 14.42), $p = 0.0598$) or MUP amplitude (patients mean = 334 μV (95% CI 294; 373) vs controls mean = 327 μV (95% CI 297; 357), $p = 0.7842$). Four patients showed signs of chronic neurogenic changes, with prolonged duration compared to controls (outside the 95% CI). Patients with prolonged MUP duration also had > 15% polyphasic potentials. One patient showed myopathic changes with short duration and small amplitude compared to controls (outside the 95% CI) (Fig. 1). Neither patients nor controls showed signs of spontaneous activity.

3.2. Muscle excitability measures

Mean surface temperature over the TA muscle was significantly higher in controls (mean = 33.52 °C (95% CI 33.15; 33.89)) compared to patients both before (mean = 32.48 °C (95% CI 32.04;

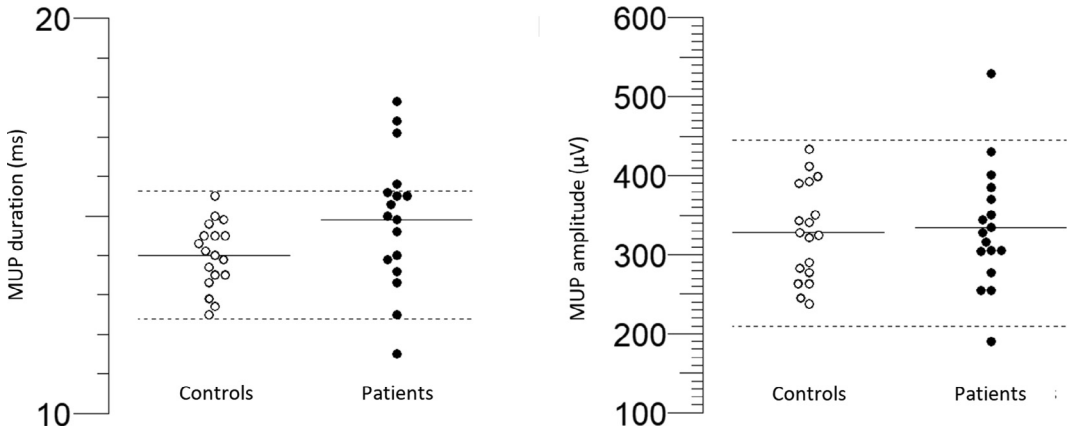

Fig. 1. Dot plots of quantitative electromyography measurements in patients with end-stage renal disease (n = 17) compared to controls (n = 18). Duration (A) and amplitude (B) of motor unit potentials (MUP). Solid lines are the mean of the group, dashed lines are 95% confidence interval for controls.

32.93), p < 0.01) and after HD (mean = 32.78 °C (95% CI 32.42; 33.14), p < 0.01). No differences were found between patients before and after HD.

3.2.1. MVRC recordings

MVRC measures are compared in Table 2. There was a shift of the curves to the right and upwards in patients before HD compared to controls (Fig. 2). This finding is reflected in a marked increase in MRRP and a decrease in the remaining parameters. After HD all the MVRC parameters were shifted towards control values, such that there was no significant difference in MVRC measures between controls and after HD (Table 2). Comparing before HD to after HD and controls, there was a significant difference (p < 0.05) in the mean of all MVRC measures (Table 2). Particularly, MRRP, ESN, 5ESN, 2XLSN, and 5XLSN, were different (p < 0.001). Adding more conditioning stimuli (2 or 5) enhanced the early and late supernormality in all subjects (Fig. 2).

3.2.2. Frequency ramp recordings

Changes in mean latencies are compared in Table 2. Two patients and one control had technically inadequate frequency ramp measurements. This was likely because of needle displacement due to muscle contractions at high frequency. In all cases, the latency measurements during the frequency ramp showed a U-shaped latency curve, with initial progressive reduction in latency, reaching a plateau before gradually increasing again (Fig. 3). Patients before HD went "round the U" more rapidly compared to both after HD and controls (Fig. 3). There was a significant reduction (p < 0.05) in all mean latencies before HD compared to both after HD and controls (Table 2). Especially, Lat(15 Hz)$_{First}$, Lat(15)$_{Last}$, Lat(30 Hz)$_{First}$, and Lat(30 Hz)$_{Last}$ were reduced (p < 0.001). There was no significant difference in mean latencies between controls and after HD recordings.

3.3. Correlation with serum constituents

Mean blood test results before and after HD are shown and compared in Table 3. All serum constituents were significantly changed (p < 0.001) after HD, except for sodium and ionized calcium. Potassium, phosphorus, chloride, creatinine, and carbamide were decreased, while pH, base excess, and bicarbonate were increased.

To explore the cause of changes in muscle excitability, excitability measures were compared with serum constituents. Correlations were first sought for measurements before HD, when there should be a stable distribution of the electrolytes between extra- and

Table 2
Comparison of muscle excitability measures.

	After vs before dialysis		Controls vs before dialysis		Controls vs after dialysis	
	mean (95% CI)	*p*	*mean (95% CI)*	*p*	*mean (95% CI)*	*p*
MVRC measures	*n = 18*		*n = 24 vs 18*		*n = 24 vs 18*	
MRRP (ms)	−1.58 (−2.08; −1.25)α	***	β	****	β	
ESN (%)	4.09 (3.03;5.14)	****	5.20 (3.65;6.76)	****	1.12 (−0.18;2.42)	
5ESN (%)	5.03 (3.75;6.31)	****	6.29 (4.66;7.92)	****	1.25 (−0.20;2.70)	
LSN (%)	0.70 (0.14;1.26)	*	1.03 (0.44;1.61)	**	0.32 (−0.38;1.03)	
2XLSN (%)	0.82 (0.50;1.13)	****	0.95 (0.57;1.33)	****	0.13 (−0.21;0.48)	
5XLSN (%)	2.87 (2.13;3.60)	****	3.17 (2.21;4.12)	****	0.30 (−0.29;0.87)	
Frequency ramp measures	*n = 16*		*n = 23 vs 16*		*n = 23 vs 16*	
Lat(15 Hz)$_{First}$ (%)	−2.07 (−3.12;−1.01)	***	−3.18 (−4.55;−1.81)	****	−1.11 (−2.42;0.21)	
Lat(15 Hz)$_{Last}$ (%)	−5.88 (−7.31;−4.44)	****	−7.94 (−9.78;−6.08)	****	−2.06 (−4.08;−0.04)	
Lat(30 Hz)$_{First}$ (%)	−4.28 (−5.79;−2.77)	****	−5.03 (−7.01;−3.06)	****	−0.75 (−2.60;1.10)	
Lat(30 Hz)$_{Last}$ (%)	−11.65 (−15.44;7.86)	****	β	****	β	
Lat(30 Hz + 30 s) (%)	−1.92 (−3.29;−0.55)	**	−1.80 (−3.36;−0.24)	*	0.11 (−1.06;1.29)	

After and before dialysis were compared using paired t-test or αWilcoxon signed-rank test. Controls and dialysis before or after were compared using unpaired t-test or βWilcoxon rank-sum test. Parametric data are presented as mean difference (95% CI) and αnon-parametric data are presented as median difference (IQR). IQR = interquartile range (1st quartile; 3rd quartile), see Figs. 2 and 3 for excitability measure abbreviations.

* p < 0.05.
** p < 0.01.
*** p < 0.001.
**** p < 0.0001.

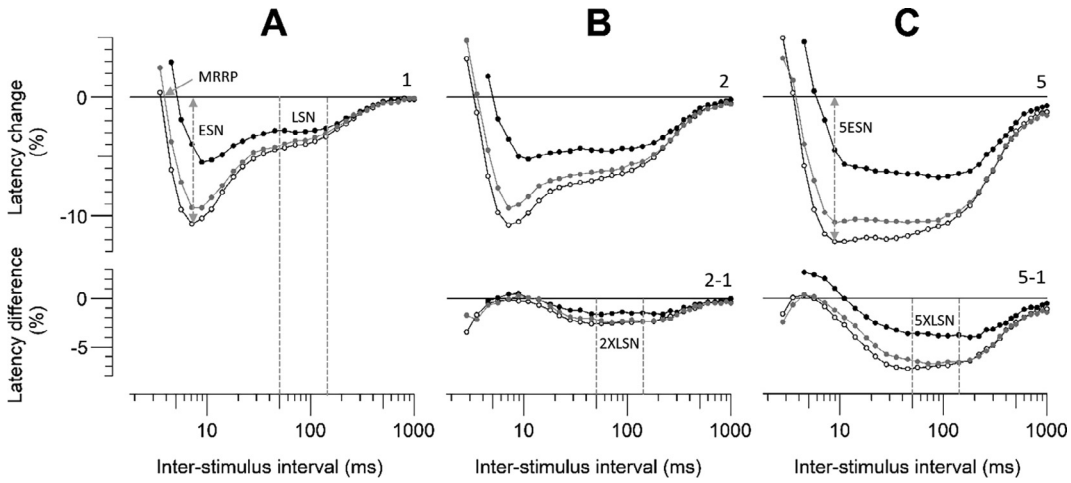

Fig. 2. Muscle velocity recovery cycles (MVRCs) in patients with end-stage renal disease (n = 18) before dialysis (black circles) and after dialysis (grey circles) compared to controls (n = 24, empty circles). *Upper:* Mean recordings of MVRCs following (A) 1, (B) 2, and (C) 5 conditioning stimuli. Percentage change in latency is plotted as a function of inter-stimulus intervals (ISIs). *Lower:* Mean latency difference between (B) 2 and 1 conditioning stimuli and (C) 5 and 1 conditioning stimuli. MRRP = muscle relative refractory period, ESN = early supernormality, 5ESN = ESN after 5 conditioning stimuli, LSN = late supernormality, 2XLSN = extra LSN for 2 conditioning stimuli, 5XLSN = extra LSN for 5 conditioning stimuli.

intracellular compartments. There was a significant correlation ($p < 0.01$) between: Potassium and MRRP ($\beta = 1.989$, 95% CI 0.63; 3.35), potassium and ESN ($\beta = -2.548$, 95% CI −4.25; −0.84), potassium and 5XLSN ($\beta = -1.615$, 95% CI −2.68; −0.55), and phosphorus and 5ESN ($\beta = -4.618$, 95% CI (−7.43; −1.81), and a significant correlation ($p < 0.05$) between: Potassium and 5ESN ($\beta = -2.075$, 95% CI −4.08; −0.07), potassium and LSN ($\beta = -0.555$, 95% CI −1.05; −0.06), potassium and Lat(15 Hz)$_{Last}$ ($\beta = 2.910$, 95% CI 0.53; 5.28) and, potassium and Lat(30 Hz)$_{Last}$ ($\beta = 8.542$, 95% CI 0.06; 17.02).

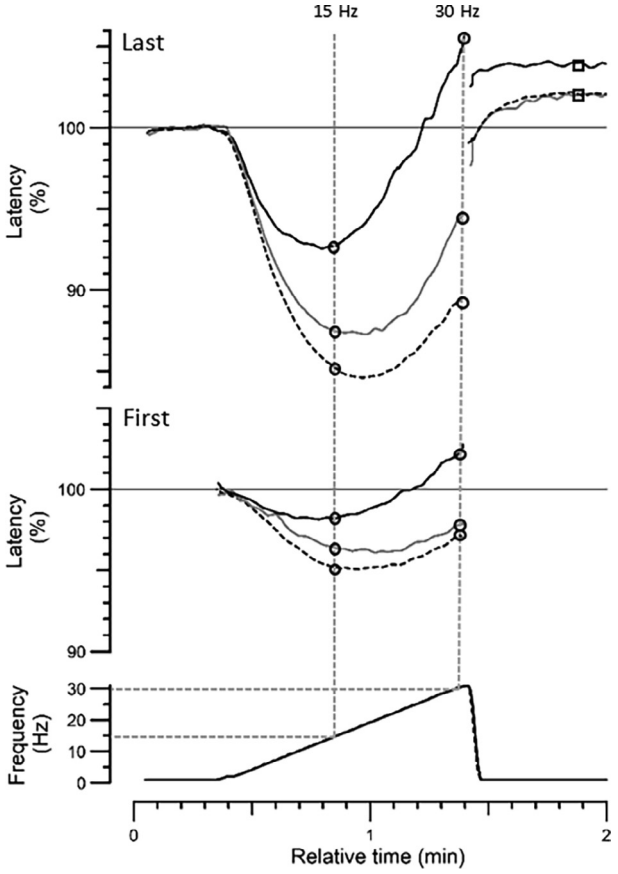

Fig. 3. Frequency ramp in patients with end-stage renal disease (n = 16) before dialysis (black lines) and after dialysis (grey lines) compared to controls (n = 23, dotted lines). Mean recordings of latency as a percentage of baseline for the last (*upper*) and first (*middle*) response in trains of 1–30 stimuli delivered in 1 s every 2 s. Small circles indicate latency change when intermittent stimulation was at 15 or 30 Hz in first or last train (Lat(15 Hz)$_{First}$, Lat(15 Hz)$_{Last}$, Lat(30 Hz)$_{First}$, Lat (30 Hz)$_{Last}$). Small squares indicate latency changes measured 30 s after end of frequency ramp (Lat(30 Hz + 30 s)). Stimulation rate during the train (*lower*).

To test further whether the changes in excitability measures caused by HD could be accounted for by changes in specific serum factors, we tested for correlation between the pooled excitability measures (before + after HD) and the corresponding serum measures (Table 4A). Changes in all excitability measures were associated with potassium ($p < 0.01$), but there were also noticeably strong associations between most excitability measures and phosphorus, creatinine, carbamide, pH, base excess, and bicarbonate (Table 4A). However, in each case this association was reduced to insignificance when correlation between potassium and serum constituents were taken into account (Table 4B).

Potassium remained significantly associated ($p < 0.05$) with MRRP, ESN, 5ESN, 5XLSN, Lat(15 Hz)$_{Last}$, and Lat(30 Hz)$_{Last}$ when adjusting for the other serum measures, except when adjusting for: carbamide (5ESN, Lat(15 Hz)$_{Last}$, Lat(30 Hz)$_{Last}$), phosphorus (5ESN, Lat(30 Hz)$_{Last}$), and creatinine (Lat(30 Hz)$_{Last}$) (Table 4C). The remaining excitability measures did not show any clear association to potassium or any other serum constituent.

We did not perform a multiple linear regression with all serum constituents included, because of the small sample size. There were no significant differences in excitability measures and serum constituents when comparing patients with PNP (n = 5) and without PNP (n = 14).

4. Discussion

This study assessed MVRCs of the TA muscle and characterized the effects of electrically induced muscle activation (frequency ramp), providing information about muscle membrane properties in ESRD patients. In summary, we demonstrated, that selected measures of muscle excitability are associated with potassium levels in ESRD patients on HD comparing measures before and after HD. Particularly, we found that hyperkalemia led to increased MRRP, Lat(15 Hz)$_{Last}$, and Lat(30 Hz)$_{Last}$ and decreased ESN, 5ESN, and 5XLSN (Fig. 4).

Table 3
Serum constituents in end-stage renal disease patients before and after dialysis.

	Before dialysis	After dialysis	After vs before dialysis	
	mean (95% CI)	*mean (95% CI)*	*mean (95% CI)*	*p*
Serum constituents	*n = 18*	*n = 18*		
Potassium (mM)	5.23 (4.89;5.58)	3.52 (3.38;3.66)	−1.75 (−1.5;−2.2)$^{\beta}$	****
Sodium (mM)	137.11 (135.74;138.49)	136.78 (135.54;138.01)	−0.33 (−2.21;1.54)	
Calcium ionized (mM)	1.17 (1.14;1.20)	1.17 (1.13;1.21)	0.00 (−0.05;0.05)	
Phosphorus (mM)	1.91 (1.70;2.12)	0.80 (0.70;0.90)	−1.11 (−1.30;−0.93)	****
Chloride (mM)	103.39 (101.33;105.44)	99.5 (98.12;100.88)	−3.89 (−5.53;−2.24)	****
Creatinine (μM)	925.61 (790.71;1060.51)	354.5 (287.31;421.69)	0.38 (0.35;0.41)$^{\alpha}$	****
Carbamide (mM)	18.82 (16.75;20.88)	6.13 (5.07;7.19)	0.32 (0.29;0.35)$^{\alpha}$	****
pH	7.38 (7.36;7.41)	7.49 (7.47;7.50)	0.10 (0.08;0.12)	****
Base excess (mM)	−2.28 (−3.98;−0.58)	4.97 (4.03;5.90)	7.25 (5.99;8.50)	****
Bicarbonate (mM)	22.62 (21.23;24.02)	29.03 (28.13;29.92)	6.41 (5.34;7.47)	****

Comparisons were made using paired t-test, presented as mean difference (95% CI) ($^{\alpha}$geometric mean x/÷ SD ratio) or $^{\beta}$ Wilcoxon signed-rank test presented as median difference (IQR). IQR = interquartile range (1st quartile; 3rd quartile).
*p < 0.05.
**p < 0.01.
*** p < 0.001.
**** p < 0.0001.

It is assumed that ESN is attributed to the passive decay of membrane charge, whereas LSN is attributed to potassium accumulation in transverse tubules (Z'Graggen and Bostock, 2009). Membrane depolarization is indicated by a prolonged MRRP and reduced early supernormality (Z'Graggen and Bostock, 2009; Z'Graggen et al., 2011), which were associated with hyperkalemia in ESRD patients. These results are consistent with Z'Graggen et al.'s findings (Z'Graggen et al., 2010). We found that HD was able to normalize all MVRC parameters, whereas Z'Graggen et al. described that HD was not adequate to normalize the late phase of supernormality (Z'Graggen et al., 2010). These discrepancies are probably due to the difference in time for the second testing. Z'Graggen et al. performed the second testing after terminating HD, whereas this study did the testing within the last 15 minutes of HD to avoid the influence of rapid redistribution of electrolytes, especially increase in plasma potassium, after terminating HD (Abuelo, 2018).

Since Z'Graggen et. al performed their study in 2010, the original MVRC protocol has been extended with 5 conditioning stimuli and a frequency ramp protocol (Boerio et al., 2012), which provides us with parameters expected to have a more pronounced association to changes in potassium level. We recorded MRVCs with trains of 5 conditioning stimuli to increase the early and late component of supernormality. ESN was strongly related to hyperkalemia both with 1 and 5 conditioning stimuli (ESN and 5ESN). On the other hand, LSN showed increasing association with hyperkalemia, when adding more conditioning stimuli, with a decrease in 5XLSN significantly associated to hyperkalemia. This is probably due to a more pronounced accumulation of potassium in the transverse tubules at 5 conditioning stimuli compared to only 1 or 2.

By adding the frequency ramp protocol, we could investigate the effects of progressive muscle activation. We found, that measures before HD went more rapidly "around the U" (Fig. 3), and an increase in Lat(15 Hz)$_{Last}$ and Lat(30 Hz)$_{Last}$ were significantly related to hyperkalemia. This is likely due to the increasing depolarizing effect of potassium accumulating intracellularly during the trains of action potentials, which, when mild, results in a reduction of latency, but with increasing frequency trains the depolarization reaches a degree, where it starts to cause sodium channel inactivation, and consequently latencies start to increase back towards baseline (Tan et al., 2014). Patients with hyperkalemia may reach the point where sodium channels are inactivated more quickly because of high potassium level intracellularly prior to examination.

It is well established that the most frequent neurological manifestation of hyperkalemia is muscle weakness (Riggs, 2002). Mild hyperkalemia can also be responsible for fatigue as a presenting symptom (Sezgin, 2020). A detailed analysis of the many factors implicated in the development of normal muscle fatigue has concluded that the dominant factor is an increase in extracellular potassium (Cairns and Lindinger, 2008). Potassium builds up during exercise, causing membrane depolarization, and animal experiments show that peak tetanic force drops precipitously when membrane potential depolarizes beyond −60 mV. Hyperkalemia accelerates fatigue by giving the rundown of the potassium gradient a head start. The multiple mechanisms by which potassium-induced membrane depolarization reduces force, inducing weakness and fatigue, mostly involving inactivation of voltage-gated sodium channels in sarcolemmal and transverse tubule membranes, are discussed further in a recent review (Lindinger and Cairns, 2021).

Our frequency ramp recordings, with latency changes going "round the U" (Fig. 3) suggestive of increased sodium channel inactivation, are consistent with this potassium hypothesis of muscle fatigue (Cairns and Lindinger, 2008; Sejersted and Sjogaard, 2000) and our new patient recordings show how this mechanism of fatigue is exacerbated by the high potassium levels in ESRD patients.

The association between muscle excitability measures and other serum factors than potassium, seen when pooling before and after HD measures (Table 4A), may indicate an additional association with acid-base balance, but all relationships turned out insignificant after adjusting for potassium. Conversely, when we adjusted the significant associations between muscle excitability measures and potassium for any other serum constituent, phosphorus, carbamide, and creatinine were able to remove the significant relationship between potassium and 5ESN, and Lat(30 Hz)$_{Last}$, respectively. This could indicate that these serum constituents also have an importance for changes in excitability measures, however, no associations were seen when pooling before and after dialysis. Only phosphorus was significantly associated to 5ESN before dialysis. We cannot exclude the possibility that other, unknown uremic toxins may also affect muscle membrane potential, e.g. by interfering with energy supply as seen in ESRD patients showing elevated resting skeletal muscle oxygen consumption and lower mitochondrial coupling ratio indicating disrupted muscle mitochondrial metabolism and uncoupling of oxidative phosphorylation (Popkov et al., 2019; Rao et al., 2018). However, the strength of the relationship with potassium indicates that any other factors

Table 4
Association between serum constituents and excitability measures in end-stage renal disease patients.

	MRRP						ESN						5ESN					
	A		B		C		A		B		C		A		B		C	
	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p
Potassium	1.297	***	-		-		−2.323	***	-		-		−2.843	***	-		-	
Sodium	0.090		0.022		1.302	***	−0.144		0.015		−2.338	***	−0.204		−0.076		−2.839	***
Calcium ionized	3.549		2.580		1.299	***	−2.467		1.366		−2.319	***	3.919		0.703		−2.836	***
Phosphorus	1.828	***	−1.286		1.711	**	−3.410	***	−0.333		−1.895	*	−4.182	***	−1.733		−1.647	
Chloride	0.214	*	0.028		1.239	***	−0.498	**	−0.114		−2.123	***	−0.49	**	−0.167		−2.530	***
Creatinine	0.004	***	−0.002		2.054	***	−0.007	***	0.003		−3.121	***	−0.008	***	0.002		−3.399	***
Carbamide	0.163	***	−0.045		1.466	*	−0.315	***	−0.047		−1.936	*	−0.386	***	−0.169		−1.602	
pH	−15.61	**	5.179		1.435	**	32.33	***	1.884		−2.074	**	37.69	***	6.85		−2.466	**
Base excess	−0.268	**	0.017		1.250	**	0.502	***	0.036		−2.129	**	0.586	***	0.158		−2.250	**
Bicarbonate	−0.297	***	0.039		1.331	**	0.571	***	0.037		−2.113	**	0.663	***	0.171		−2.284	**
	LSN						2XLSN						5XLSN					
	A		B		C		A		B		C		A		B		C	
	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p
Potassium	−0.445	**	-		-		−0.438	***	-		-		−1.622	***	-		-	
Sodium	−0.059		−0.036		−0.434	*	−0.032		0.001		−0.438	***	−0.155		−0.060		−1.606	***
Calcium ionized	−1.138		−1.134		−0.446	**	−2.254		−1.692		−0.436	***	−6.960		−5.664		−1.617	***
Phosphorus	−0.663	**	−0.331		−0.249		−0.621	**	−0.124		−0.361		−2.171	***	0.522		−1.659	***
Chloride	−0.101		−0.028		−0.383	*	−0.062		−0.006		−0.426	**	−0.181		−0.013		−1.581	***
Creatinine	−0.001		0.001		−0.673	*	−0.001	**	0.0001		−0.425	*	−0.005	***	0.001		−1.859	***
Carbamide	−0.059	**	−0.015		−0.337		−0.064	***	−0.049		−0.075		−0.221	***	−0.064		−1.187	*
pH	4.920		−2.680		−0.586	*	5.672	**	0.552		−0.393	*	18.99	***	−3.159		−1.725	***
Base excess	0.088	*	−0.001		−0.430		0.098	**	0.045		−0.255		0.327	***	0.029		−1.458	**
Bicarbonate	0.100	*	0.005		−0.480		0.109	***	0.044		−0.281		0.370	***	0.024		−1.480	***
	$Lat(15\ Hz)_{First}$						$Lat(15\ Hz)_{Last}$						$Lat(30\ Hz)_{First}$					
	A		B		C		A		B		C		A		B		C	
	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p
Potassium	1.158	**	-		-		3.281	***	-		-		2.362	***	-		-	
Sodium	0.006		−0.047		1.171	**	−0.094		−0.325		3.337	***	−0.233		−0.293		2.440	***
Calcium ionized	−0.604		−2.541		1.159	**	−7.260		−12.96		3.245	***	−11.31		−10.16		2.363	***
Phosphorus	1.581	**	0.063		0.910		4.670	***	0.950		2.709	*	3.797	***	2.661		0.789	
Chloride	0.234	*	0.025		1.096	**	0.471		−0.083		3.454	***	0.402		−0.091		2.624	***
Creatinine	0.003	**	0.0002		0.940		0.009	***	0.0003		3.189	**	0.007	***	0.003		1.254	
Carbamide	0.161	**	0.086		0.676		0.446	***	0.136		2.342		0.326	***	0.113		1.620	
pH	−15.03	**	2.529		1.275	*	−44.85	***	5.982		3.611	***	−30.55	**	6.787		2.797	**
Base excess	−0.287	**	−0.148		0.860		−0.772	***	0.021		3.360	**	−0.562	***	−0.010		2.439	*
Bicarbonate	−0.288	**	−0.068		0.921		−0.794	***	−0.002		3.414	**	−0.566	***	0.005		2.508	*
	$Lat(30Hz)_{Last}$						Lat(30Hz+30s)					
	A		B		C		A		B		C	
	β	p	β	p	β	p	β	p	β	p	β	p
Potassium	6.434	***	–		–		1.000	**	–		–	
Sodium	−0.121		−0.387		6.583	***	−0.179		−0.201		1.028	**
Calcium ionized	−25.98		−19.66		6.406	***	−8.981		−7.636		0.996	**
Phosphorus	N/A		5.396		4.234		1.691	**	1.201		0.173	
Chloride	1.288	*	−0.090		6.964	***	0.144		−0.015		1.013	*
Creatinine	0.020	***	0.001		4.708		0.003	**	0.002		−0.374	
Carbamide	0.910	***	−0.108		6.444		0.156	**	0.189		−0.773	
pH	−79.14	**	33.197		8.234	**	−12.58		−0.861		0.956	
Base excess	−1.458	**	0.304		7.859	**	−0.255	*	−0.169		0.514	
Bicarbonate	−1.495	***	0.378		7.970	**	−0.245	*	−0.118		0.562	

Mixed linear regression was performed and presented as slope coefficient (β). **(A)** Pooled associations (before + after dialysis), **(B)** and after adjusting for relationship of the serum constituents with potassium, **(C)** and after adjusting for relationship of potassium with any other serum constituent. See Figs. 2 and 3 for excitability measure abbreviations, N/A = not applicable.

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

are probably of minor importance. In addition, HD is known to "over-correct" the composition of electrolytes resulting in more alkalotic and lower potassium concentration in the end of HD than normal people.

We did not find significant changes in EMG parameters; only one patient showed signs of myopathic changes, and no patients showed clinical muscle weakness. However, the described feeling of increased fatigue, limited endurance, and exercise limitation could probably be due to the changes in muscle excitability measures due to high potassium levels in the blood. These reversible functional changes (Z'Graggen et al., 2010), may be early signs of uremic myopathy which suggests that MVRC and frequency ramp could be used to detect early changes of disease, before detectable with conventional electrophysiological methods and occurrence of structural changes in biopsies.

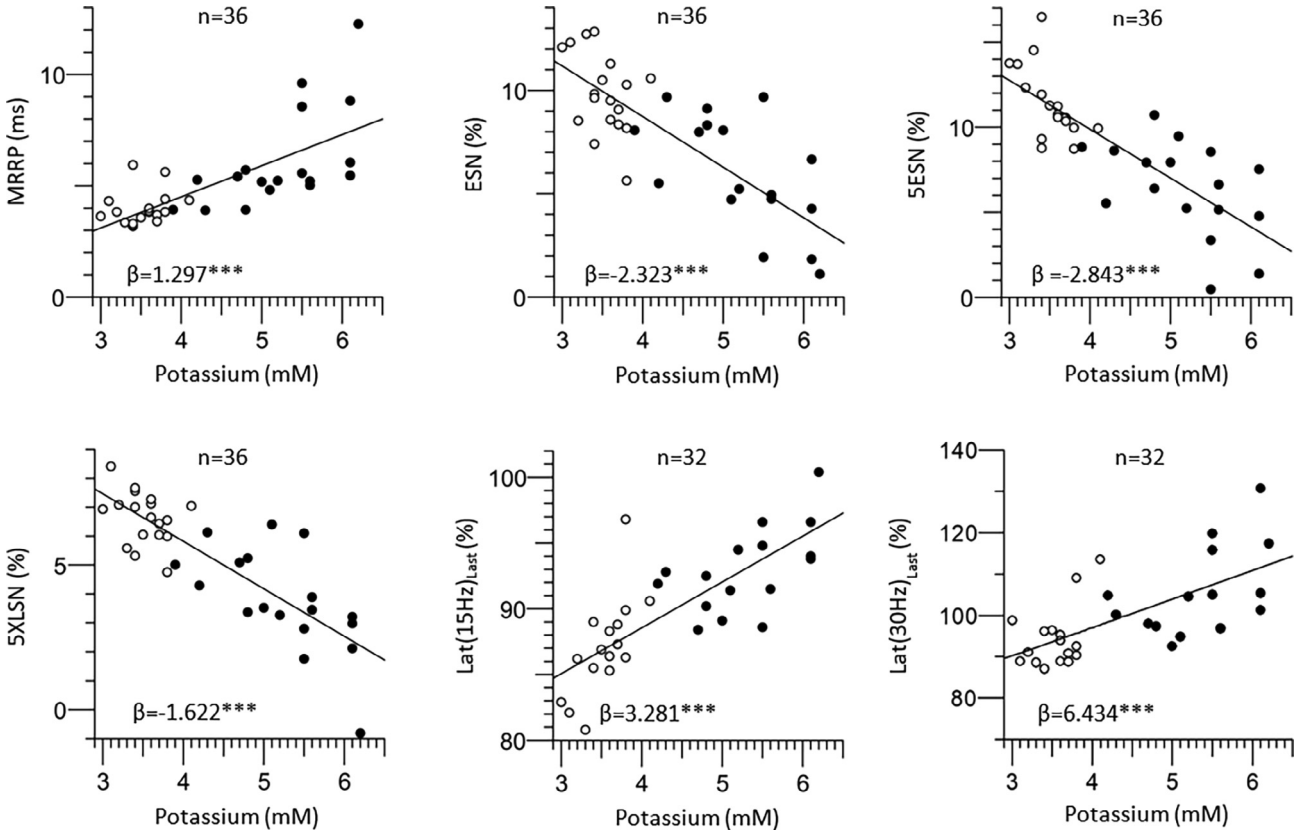

Fig. 4. Unadjusted significant relationship between muscle excitability measures and potassium levels in combined patient recordings (before + after dialysis). Regression lines and slope coefficients (β) are for all 36(32) recordings, comprising 18(16) before dialysis (filled circles) and 18(16) after dialysis (empty circles). See Figs. 2 and 3 for excitability measure abbreviations, ***=p < 0.001.

We have seen that there is no simple clinical test for uremic myopathy: the primary symptom is muscle weakness, but in most cases the only clinical symptom is the relatively non-specific one of muscle fatigue. Here we consider whether that the membrane depolarization by hyperkalemia, for which our muscle recordings provide good evidence, can help explain these symptoms.

The idea that muscle membrane depolarization is the crucial factor in uremic toxicity was proposed by Cotton et al. (Cotton et al., 1979), who recorded membrane potential directly with glass microelectrodes (Cunningham et al., 1971). In 9 ESRD patients they found that a mean muscle fiber resting potential of −78.5 mV was improved to −87.8 mV after 7 weeks of HD, and in another 7 patients on regular HD they found that lowering the frequency from 6 hours thrice weekly to 6 hours twice weekly caused muscle membranes to depolarize from −90.2 to −80.1 mV with consequent increase in symptoms of general malaise and fatigue. They proposed serial membrane potential measurements as a powerful tool to assess adequacy of dialysis, but their method was inconvenient and was not followed up. The MVRC method has amply confirmed the findings on muscle membrane potential changes by more convenient, though indirect, means, and also provided good evidence that much the most important cause of membrane depolarization is hyperkalemia.

4.1. Limitations

We were only able to include a relatively small sample size, making it impossible to include all parameters in a multiple regression, which would be preferable in assessing associations between excitability measures and all serum constituents. Muscle excitability parameters in particular MRRP and ESN are temperature-dependent (Bostock et al., 2012), and the significant difference in temperature between patients and controls may account for some of the difference seen in excitability measurements. However, there was no temperature difference before and after HD for which we found the most prominent findings. We examined the anterior tibial muscle, since its well-defined end-plate zone makes it particularly suitable for MVRC recordings. However, to clarify the relationship between the changes revealed by this study and uremic neuropathy it will be necessary to extend the recordings to proximal muscles, which are more prone to the disease. It will also be important to test for a relationship between hyperkalemia and the structural, histochemical and immunohistological changes revealed by muscle biopsy. Another main limitation of our study is that we could only discuss the role of serum constituents in uremic myopathy. Future studies should address additional possible mechanisms such as uremic sarcopenia/myopathy, possibly resulting from imbalance between muscle protein synthesis and catabolism (Nishi et al., 2020) and mitochondrial dysfunction (Liu et al., 2020).

4.2. Conclusion

This study suggests that muscle membrane in ESRD patients is depolarized, mainly due to hyperkalemia. This has been supported with pronounced changes in MVRC and frequency ramp parameters as well as the relationships with serum constituents. It is argued that this potassium-induced depolarization resembles that in fatigued muscle and is likely to be a major cause of uremic myopathy in ESRD patients on HD therapy.

Declaration of Competing Interest

Professor Hugh Bostock receives royalties from UCL for sales of his Qtrac software used in this study. The other 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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