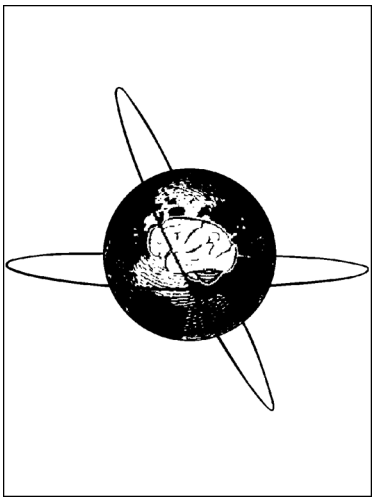

Fibromyalgia: increased reactivity of the muscle membrane and a role of central regulation

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Fibromyalgia: increased reactivity of the muscle membrane and a role of central regulation

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Highlights:

Muscle fiber conduction in fibromyalgia is precipitated by changed membrane physiology.

Muscle membrane conduction speed in fibromyalgia rises excessively when adopting a limb position.

The muscle membrane in fibromyalgia is probably hyperactive due to deregulation from higher systems.

Abstract

Objective: Fibromyalgia (FM) is characterized by widespread muscle pain and central neural deregulation. Previous studies showed increased muscle fiber conduction velocity (CV) in non-painful muscles of FM patients. This study investigates the relationship between central activation and the CV in FM.

Methods: Twenty-two females with primary FM and 21 controls underwent surface electromyography of the non-painful biceps brachii. Mean CVs were calculated from the motor unit potential velocities (CV-MUPs), and the CV-MUPs' statistical distributions were presented as histograms. The amount of muscle activity (average rectified voltage, ARV) was measured.

Results: The CV was higher in the FM-group than in the controls (P=0.021), with CV-MUPs generally shifted to higher values, indicative of increased muscle membrane propagation speeds. The largest increase in the CV of the FM-group occurred when adopting and maintaining a limb position at only 5% of maximum strength (P<0.001); the CV did not, as normal, increase with greater force. However, the ARV in both groups similarly increased with force.

Conclusions: In fibromyalgia patients, the muscle membrane propagation speed increases independently of the force load or amount of muscle activity produced. When adopting a limb position, the patients show an augmented muscle membrane reaction, suggesting deregulation from higher neural centers.

Significance: These findings contribute to understanding fibromyalgia.

Keywords: fibromyalgia; surface electromyography; muscle fiber conduction velocity; motor unit action potential; muscle membrane; central activation.

Abbreviations:

sEMG, surface electromyography

CV, mean muscle fiber conduction velocity

MUP, motor unit action potential

CV-MUP, conduction velocity of a motor unit action potential

ARV, average rectified voltage or sEMG amplitude; a measure of intensity of motor unit activity

1. Introduction

Fibromyalgia (FM) is a chronic pain condition of unknown etiology and unclear pathophysiology. FM is characterized by widespread, especially tendomuscular, pain and generalized hypersensitivity to pain (Mease, 2005; Wolfe and Rasker, 2013; Yunus, 2008). There is evidence of hyperactivation/deregulation at various levels of the central nervous system (Banic et al., 2004; Burgmer et al., 2012; Choi et al., 2016; Desmeules et al., 2003; Gracely et al., 2002; Truini et al., 2015; Yunus, 2008). In addition, there is growing evidence of muscular function disturbance (Ge et al., 2009; Hubbard and Berkoff, 1993; Klaver-Krol, 2017; Vitali et al., 1989). A notable phenomenon is the increased muscle fiber conduction velocity (CV) that occurs not only in painful (Gerdle et al., 2008), but also in non-painful muscles of FM patients (Casale et al., 2009; Klaver-Krol et al., 2012). This suggests that the increased CV is not a local muscle problem, e.g. due to local changes in histopathology and microcirculation (Gerdle et al., 2008), but rather a generalized muscle phenomenon. If an aspecific local muscle microdamage, as it occurs in FM patients (Bengtsson et al., 1986), results in thicker muscle fibers, this would lead to a CV increase because thicker fibers conduct an action potential faster than thin ones (Blijham et al., 2006). The evidence of regulatory disturbance in various central neural circuits in FM makes

it conceivable that regulation in the efferent motor system might also be disturbed, influencing the CV.

The goal of the present study was to investigate the relationship between central regulation and CV in patients with FM. Specifically, we searched for possible abnormalities in the changes in CV related to force load, abnormalities in the amount of muscle activity produced, and we investigated the relationship between the CV and the amount of produced activity.

To achieve the above goals, we measured the CV by surface electromyography (sEMG) using an elaborated method previously developed to distinguish between the muscles of sprinters and of long-distance runners (Klaver-Krol et al., 2010). From the sEMG signals, large sets of motor unit action potentials' velocities (MUPs, CV-MUPs) were obtained. From these CV-MUPs, we calculated not only the average CV, but we were also able to present histograms of the statistical distribution of the CV-MUPs. This method enables us to extrapolate the findings related to MUP velocities towards motor unit recruitment (Klaver-Krol et al., 2007). This approach helps to distinguish between the recruitment and the muscle membrane problem as a cause of increased CV. Parallel with the CV, the amount of muscle activity produced was measured as the average rectified voltage (ARV). The ARV provides information about the total amount of recruitment and the firing frequency of the motoneurons involved. Indirectly, it tells us about the synaptic input from the central drive and spinal reflexes to the motoneurons in a given situation and, thus, it tells us about the central neural regulation.

The data were obtained from a clinically unaffected (non-painful) biceps brachii muscle of females with primary FM (i.e. no concurrent diseases) (Yunus et al., 1981) and of healthy controls. We applied a range of forces under static conditions. We chose static circumstances

because these require a high level of central control (Mottram et al., 2005), which may elicit control imbalance in FM.

2. Methods

2.1. Study participants

Twenty-two female patients with primary FM (Yunus et al., 1981) (FM subjects) and 21 female healthy controls (controls) volunteered for the study. The FM subjects were recruited via the website of the Dutch Fibromyalgia Association. The controls were healthy friends or neighbors of the FM subjects. The groups were matched for sex, age, body posture (height and weight) and level of physical activity . Prior to the enrollment in the study, all potential participants were screened by telephone and/or e-mail to ensure if they met basic requirements. Further, all subjects underwent a physical examination. Those with abnormalities unrelated to FM or with findings disturbing the performance during the experimental procedure were excluded from the study. The inclusion criteria for the patients were: primary FM (no concurrent rheumatologic disease) (Yunus et al., 1981), fulfilling the 1990 American College of Rheumatology (ACR) diagnostic criteria for FM (Wolfe et al., 1990), diagnosed by a rheumatologist, female, age 18 to 75 years, and duration of symptoms of at least two years. Exclusion criteria specific for the FM patients were: severe disablement requiring the uses of orthoses and/or a wheelchair (Klaver-Krol et al., 2012). Patients involved in legal procedures in respect to employment or disability were also excluded.

Exclusion criteria for all subjects were: pain in the shoulder, elbow or wrist of the dominant arm (because this would disturb the test performance); obesity (a body mass index > 28); diabetes mellitus, malignancy, cardiovascular, lung or renal diseases, hypothyroidism, hyperthyroidism, myopathy and neuropathy (because these diseases can affect CV results) (Klaver-Krol et al.,

2012). Using medicines such as β-blockers, muscle relaxants and narcotics, using magnesium holding supplements (because Mg affects the neuromuscular transmission), and substance abuse were also reasons for exclusion. Because we chose to investigate a clinically unaffected biceps brachii muscle, subjects were excluded which, at examination, had palpation pain in the biceps brachii. Subjects were also excluded when the skin thickness on the spot where the electrodes were to be placed exceeded 10.0 mm, because a thick skin layer under the electrodes influences the CV estimates (Hogrel et al., 1998).

The protocol was conducted in accordance with the Helsinki Declaration of the World Medical Association and was approved by the local ethics committee (METC Twente, Enschede, The Netherlands). A written informed consent was given by all participants.

2.2. Experimental session

The experimental sessions always took place at the same time, in the afternoon, in order to prevent different influences of the circadian rhythm on the muscle performance between the subjects (Martin et al., 1999). The subjects were required to take a light lunch at least one hour before the session. It was also required not to use caffeine-holding substances for at least two hours before the session because of a possible influence on the CV results (Islam et al., 1995), and not to use tobacco for one hour before the session.

2.2.1. Measurements of the self-reported pain and of muscle tenderness

All participants completed a visual analogue scale for their pain severity on the day of experiment. The VAS-pain scores range from 0 to 10 cm (0 = no pain, and 10 = worst pain imaginable).

The measurements of tenderness were performed prior to the sEMG measurements by the same experienced observer (MK), blinded as to the condition of the participants. The observer examined each participant by manual palpation of the 18 standardized body sites, the tender points (TPs) (Klaver-Krol et al., 2012), as defined in the 1990 ACR criteria for FM (Wolfe et al., 1990). TPs are situated at the musculotendinous junctions, especially in the supporting and extensor muscles (Ge et al., 2010; Jacobs et al., 1995; Wolfe et al., 1992). The TP score was calculated both a) as the TP number = a number of sites where the subjects stated that the palpation was painful (range 0 to 18), and b) as a total TP pain intensity score = sum of pain intensities from all TPs, on a scale of 4 points (range 0 to 54) (Dunkl et al., 2000).

2.2.2. Surface EMG measurements

Set-up. In the set-up of the sEMG measurements, we tried to mimic natural everyday activities: while applying static conditions, the force was exerted by an inertial load put in the palm of the hand. The methods have been described in detail previously (Klaver-Krol et al., 2007; Klaver-Krol et al., 2010). Maximum voluntary contraction force (MVC = maximum strength) of the elbow flexors was measured with a hand-held dynamometer (Lameris Instruments, Utrecht, The Netherlands). During the MVC measurements the subjects were seated upright. The shoulder was slightly abducted and flexed at 45˚; the elbow was firmly supported and flexed at 90˚, while the forearm was supinated. The dynamometer was applied to the wrist (van der Ploeg et al., 1991).

During the experiment, the subjects were seated in a chair. The upper arm was slightly abducted and comfortably supported at 45˚ of shoulder flexion; the forearm was free and supinated. Subjects were required to hold the forearm horizontally (at the elbow angle of 135˚). An adjustable horizontal bar was used that showed the subjects the position at which the lower arm was really horizontal (Klaver-Krol et al., 2010). The position was held for 6 – 7 seconds; the

measurements were performed during 4 seconds. Four force levels were applied in blocks that were three minutes apart: unloaded, 5%, 10% and 20% MVC. Every block/test was made up of three sub-tests (three repetitions at the same force level) that were separated 30 s from one another (Klaver-Krol et al., 2010). In the loaded tests a bag with sand and lead was put in the palm. For the unloaded test, the requirement was "Direct your fingers towards the target (at the horizontal bar), with the palm up, and hold the arm relaxed". For the loaded test it was: "Prepare the position by directing the fingers towards the target, with the palm up. A weight will be put on your palm. Keep then the arm correctly, without lowering the weighted arm".

EMG recording and data processing. Measurements were performed on the short head of the biceps brachii of a dominant arm (Klaver-Krol et al., 2007). A surface electrode array consisted of three gold-coated electrodes (Harwin, P25-3526), diameter 1.5 mm and the inter-electrode distance of 15 mm (Klaver-Krol et al., 2012). The electrode array was placed parallel to the muscle fibers (Hogrel et al., 1998). Bipolar derivation was done, resulting in two differential sEMG signals. A correlation coefficient between the signals was accepted at $r > 0.7$ (Hogrel et al., 1998; Merletti et al., 2001). The signals were amplified (gain 2,000 to 10,000 times) and band-pass filtered at 2-250 Hz by an EMG apparatus (Viking IV, US). The signals were digitized and stored on a PC (sampling 10 kHz, 12 bits acquisition). Data were analyzed with LabVIEW version 6.1.

The skin temperature was measured with an electronic thermometer (NTC type, Viking, US), the sensor was placed about 5 cm proximally from the derivation electrode. The skin thickness was measured with calipers, on the spot where the electrodes were placed (Klaver-Krol et al., 2010).

2.2.3. The peak selection and sEMG variables

The CV calculation was based on the inter-peak latency (IPL) method that was first described by Lange and colleagues (Lange et al., 2002), and later modified by our group (Klaver-Krol et al., 2007; Klaver-Krol et al., 2010). The principle of the IPL method is to measure conduction velocities between identical motor unit action potential's (MUP) elements (peaks) of two, or more, EMG signals derived in the length of muscle fibers.

The peak selection algorithm. The algorithm of MUP/peak selection and of calculation of the MUP/peak velocities, as used in the present study, is visualized in Fig. 1. The peaks were detected over non-overlapping 200 ms epochs for each of the two differential signals obtained along the muscle fibers separately.

Step 1: Finding the highest MUP within a 200 ms epoch, and determining 20% of its peak-to peak amplitude (Fig. 1A).

Step 2: Searching declines. A decline is a consecutive diminution of a potential over a period of at least 40 data-points, which is 4 ms, and is searched with a moving time window (Fig.1B). A decline has to be at least 20% of the peak-to peak amplitude of the highest MUP in a 200 ms epoch. A decline corresponds with repolarization phase of a MUP.

Step 3: Determining peaks. A peak is the highest point prior to a decline (Fig. 1C). The peaks are turning points between depolarization and repolarization phase. In Fig. 1, the peaks are upwards directed.

Step 4. Finding pairs of peaks between the two signals (Fig. 1C). Every peak in the first signal has an unique paired peak in the second signal. A paired peak in the second signal is searched with a time window of 2 to 6 ms, which corresponds with the expected MUP propagation

velocities of 2.5 to 7 m/s. If there are two peaks within a period of 6 ms, the second peak must be excluded in order to avoid double calculations of the inter-peak intervals for the same peaks.

Step 5. Calculating MUP/peak propagation velocities (CV-MUPs). From the inter-electrode distance (15 mm) and the time delays between paired peaks, the CV-MUPs are calculated.

The sEMG variables. The following sEMG variables were used:

1. The mean muscle fiber conduction velocity (CV), which is an average of the CV-MUPs at a given force level (Klaver-Krol et al., 2007; Lange et al., 2002). First, a set of CV-MUPs was obtained for every of the three sub-tests at a given force level. Then, the three CV-MUP sets of a given force level were taken together to form a large set from which the final CV value was calculated. In a sub-test, the MUPs were extracted over a period of 4 s (the duration of the measurement). The 4-second data contains 20 linked non-overlapping epochs of 0.2 seconds.

2. The average rectified voltage (ARV) or sEMG amplitude. The AVR is an often used measure of intensity of motor unit activity, expressed as area under the curve of a sEMG signal. ARV is a relative measure that shows the change in the sEMG amplitude in respect to the basic value, here the ARV value in the unloaded test. As for CV, the ARV is calculated over periods of 4 s in 20 linked non-overlapping epochs of 0.2 s. A total ARV of a given force level is a sum of three 4 s periods (the three sub-tests).

2.3. Statistical analysis

For analyses of the CV and the ARV, repeated measures of ANOVA were applied (Kutner, 2005) that included the within-subjects factor ‘force’ at 4 levels (unloaded, 5%, 10% and 20% of MVC) and the between-subjects factor ‘group’ at two levels (FM subjects and controls) (Klaver-Krol et al., 2010). If significant interactions were observed between the factors force and group, post-hoc

analyses were applied for every group using the factor 'force'. To calculate associations between variables, Pearson's correlation coefficients were used. If needed, paired or independent *t*-tests were applied. The analyses were performed using SPSS 22.0 software. The statistical significance level was set at $P < 0.05$, two-tailed.

3. Results

3.1. Participant characteristics and muscle tenderness

Characteristics of the FM subjects and controls are summarized in Table 1. The strength of FM subjects was 8% lower than that of controls ($P = 0.047$). Both the number of TPs and the total TP pain intensity score were much higher in FM subjects than in controls (for both $P < 0.001$). The self-reported pain on the VAS was also significantly greater in FM subjects than in controls ($P < 0.001$).

3.2. Surface EMG

Mean muscle fiber conduction velocity (CV). Over the four tests (unloaded, 5%, 10% and 20% MVC), the CV was significantly higher in FM subjects than in controls ($P = 0.021$). The results are presented in Fig. 2 A1. The largest difference between groups was at 5% MVC (unloaded $P = 0.086$; 5% MVC $P = 0.006$; 10% MVC $P = 0.022$ and 20% MVC $P = 0.049$).

The course of the changes in the CV on force differed between groups, especially at very low force levels (over the four tests, $P = 0.125$; over the Unloaded, 5% and 10% MVC tests, $P = 0.015$). The mean CV of the controls increased slightly, regularly, but non-significantly, with force (changes on force over the four tests, $P = 0.902$), whereas the CV of FM subjects increased sharply in the 5% MVC test compared with the preceding Unloaded test ("CV jump"). This increase was 0.13 $m \cdot s^{-1}$ ($P < 0.001$), see Fig. 2 A1. In the following tests with 10% and 20%

MVC, the CV of FM subjects did not increase further but declined slightly; however, this decline was statistically not significant (5% vs 10%, $P = 0.236$; 5% vs 20%, $P = 0.249$; 10% vs 20% MVC, $P = 0.461$). Controls had no sharp CV increase between the Unloaded and 5% MVC test ($P = 0.986$).

Average Rectified Voltage (ARV). Overall, the ARV increased significantly with force ($P < 0.001$), see Fig. 2 B1. The greatest increase appeared between the Unloaded and 5% MVC test (Unloaded vs 5% MVC $P < 0.001$; 5% MVC vs 10% MVC $P = 0.529$; 10% vs 20% MVC $P = 0.041$). The course of the ARV on force did not differ between FM group and controls ($P = 0.897$). Specifically, the changes between Unloaded and 5% MVC test were similar in the two groups ($P = 0.852$).

Histograms. In FM subjects, the histograms of CV-MUPs showed a shift towards higher values over their whole range (see an example of a characteristic FM subject and a control person in Fig. 3). At all force levels, there were only few of the slow CV-MUPs in FM subjects compared with controls (Fig. 3A, the 'a' arrows). FM subjects showed also more of the fast propagating MUPs than controls (Fig. 3A, the 'b' arrows). Furthermore, the velocities of these fast propagating MUPs were very high.

3.3. Correlations between sEMG findings, muscle tenderness and other relevant items

In FM subjects, there was a positive correlation between the CV and muscle tenderness (for the total TP pain intensity score: between $r = 305$, n.s. in the Unloaded test and $r = 0.693$, $P < 0.001$ at the "CV jump"; for the TP number: statistically significant only at the "CV jump", $r = 0.464$, $P = 0.039$).

No significant correlations were observed between the CV and ARV of FM subjects (ARV cumulative between r = -0.088 for the 20% MVC test and r = -0.227 for the 5% MVC test). No significant correlation were found between ARV and variables of the muscle tenderness of FM subjects (ARV cumulative between r = -0.084 for the TP number and r = -0.064 for the total TP pain intensity).

No significant correlations were found between the CVs and the self-reported pain of FM subjects (VAS-pain vs CV cumulative r = -0.318, P = 0.149; VAS-pain vs CV jump r = -0.227, P = 0.310). Also no significant correlation was found between the TP pain intensity and the self-reported pain (r = -0.20, P = 0.399).

No significant correlations were found between age, body mass, height, muscle strength or duration of symptoms and TP pain intensity (between r = -0.016, P = 0.945 for strength and r = 0.251, P = 0.260 for age).

4. Discussion

The present study shows that the increased CV in patients with FM is unrelated to the increase in the force load and the amount of muscle activity produced. The conduction velocities of the patients' motor unit action potentials are generally shifted to higher values, which is indicative of increased muscle membrane conduction speed. The CV of FM subjects seems to rise in a situation where a limb position is adopted and rigidly maintained.

Increases in CV could reflect changes in motor unit recruitment or in the muscle membrane. If the cause is linked to recruitment, the CV would increase with both the force load and the amount of muscle activity since the large, high discharge-threshold motor units with their fast propagating muscle fibers are activated at higher forces (Henneman et al., 1965). Since the CV of

FM subjects in our study did not increase with either force or the amount of muscle activity produced, the results suggest that the CV increase was predominantly linked to the membrane state. Histograms of the motor unit action potential velocities of FM patients support this inference: the MUP velocities are as a whole shifted to higher values. That is, there are only a few slow propagating MUPs within the MUP population in the muscles of FM subjects. This suggests that their low-threshold (tonic) motor units, which basically propagate slowly, conduct their action potentials relatively fast along the muscle membranes. FM subjects also have many MUPs with very high velocities, meaning that their higher-threshold (intermediate) motor units, with their primarily fast propagating muscle fibers conduct faster than normal along their membranes. Altogether, the propagation velocities of FM patients seem to be higher across all muscle fibers, irrespective of their type, indicative of overall disturbed membranes. Recruitment probably plays a relatively minor role in the CV increase.

Previous studies have found a form of hyperactivity/hypersensitivity occurring at various levels of the nervous system in FM patients (Banic et al., 2004; Burgmer et al., 2012; Choi et al., 2016; Yunus, 2008) and the question arises if there is also a central efferent deregulation that would disturb muscle membrane function. Intuitively, it is easy to conceive that a deregulation/ hyperactivation in the higher neural centers would increase motoneuron activity, leading to intensified recruitment of the high-threshold neurons. However, the muscle membrane itself can also become hyperactivated by an augmented central activation, with increased CV as a result. Such an effect can occur due to depolarization of the muscle membrane, either accompanied by overt muscle activity, or without such activity: through subthreshold muscle stimulation from the motoneurons. Conduction along the membrane depends largely on its resting potential (Buchthal and Sten-Knudsen, 1959; Kernell, 2006). A resting membrane is intracellularly negative.

Reducing this intracellular negativity makes the *membrane more excitable* and increases its conduction speed (Ganong, 1979; Kernell, 2006) such that the membrane becomes hyperactivated/facilitated (Buchthal et al., 1955). Following muscle activation, the membrane potential becomes less negative (the after-depolarization) (Bergmans, 1971) and this, depending on the intensity of the stimulation or voluntary activation, may remain for minutes or even hours, and results in a long-lasting high CV (Gydikov and Christova, 1984; Van der Hoeven and Lange, 1994). It has been demonstrated that, when performing a series of voluntary contractions, FM patients produce significantly more non-intended muscle activity between the contractions than the control subjects. The FM patients also have difficulty in relaxing their muscles after performing a task (Elert et al., 1989). This might indicate both augmented central activation and augmented muscle membrane reaction during the after-depolarization phase in FM. The hypothesis of a disturbed/hyperactivated muscle membrane in FM is also supported by evidence that the muscles of patients, when under ischemic conditions, show signs of "neuromuscular hyperexcitability" in the form of spontaneous membrane discharges (Vitali et al., 1989).

In our experiments, the amount of muscle activity (the ARV) produced during the tests did not significantly differ between FM subjects and controls. This suggests that the FM membranes might have become hyperactivated through *subthreshold stimulation* from the motoneurons. Subthreshold stimuli that do not produce an action potential lead to a localized depolarizing potential change that decays exponentially with time (Ganong, 1979). In chemical synapses, such as the neuromuscular junction, a spontaneous increase in the release of transmitter quanta (membrane noise or end-plate noise), even in an absence of a postsynaptic action potential, would change the average membrane potential level (Hubbard et al., 1967). There is supporting evidence for a subthreshold stimulation of the muscle in FM patients in that in patients with myofascial pain syndrome, a condition that overlaps with FM, the prevalence of end-plate noise

is higher than in healthy controls. Further, a high correlation was found between the level of end-plate noise and the pain threshold and pain intensity (Kuan et al., 2007; Simons, 2001).

In the present study, the largest increase in the CV of the FM subjects occurred at a force of only 5% MVC, applied following a static test in an unloaded situation. This "jump" in CV is remarkable given this near-negligible force and, further, at higher forces, the CV did not increase further and sometimes even declined. Interestingly, the amount of sEMG activity increased equally in both the FM and control groups, and much more between the no-load and low-load situations than between the larger loads that followed. This suggests that transitioning between the no-load and loaded situations may place high adaptive demands on the motor system, with a large synaptic input into the motoneuron pool. Consequently, the simplest explanation of the "CV jump" in FM subjects could be that it represents their augmented reaction to a transition between no-load and loaded situations. However, in an earlier study, we found that the highest CV did not appear at 5% MVC but in the initial unloaded situation when asked only to maintain a position, see Fig. 2 A2, (Klaver-Krol et al., 2012). Given that, in the previous study, the high CV in the unloaded test was not accompanied by an increased ARV but, consistent with the present study, the increase in ARV occurred predominantly at the transition between the no-load and loaded situations, indicates that the increase in CV cannot be explained by the "transition". The difference in the results could be due to differences in how the requirements for the unloaded test were verbalized in the two studies: in the earlier study, the emphasis was on maintaining the arm position secure and rigid (which requires mental focusing) whereas, in the present study, the subjects were asked to keep the arm loose. In these present tests, a requirement to maintain the arm position secure was only applied for the 5% MVC and consecutive loading tests. The combined outcomes of the two studies suggest that the phenomenon of increased CV in patients

with FM may be linked to *mental alertness*/mental focusing when *adopting and maintaining* a given *position in static circumstances, and especially at low force levels.* The findings resemble an increased stress reaction in the motor system, followed by an adaptation.

The motor system of FM patients may be hyperactive due to either increased facilitation or lack of inhibition, as it is in their sensory systems (Jensen et al., 2009; Kosek et al., 1996; Truini et al., 2016). When a muscle reacts to adopting a position, the gamma-loop, with its gamma-motoneurons in the spinal cord, the muscle spindles, and their afferents that make direct connections with the alpha-motoneurons, all play important roles. The spindles provide information about muscle lengthening when a position is adopted or a force applied, and the gamma-loop continuously makes facilitatory or inhibitory adjustments to the alpha-motoneuron activity, known as the alpha-gamma linkage (Granit, 1977). The alpha- and gamma- motoneurons are, in turn, activated and controlled by the higher regulatory centers. Adopting and maintaining a position requires both control of the body's balance and visual control. As such, it seems likely that, in our setting, several *supraspinal*, cerebral and cerebellar structures influence the gamma-neurons, and may excessively augment the spontaneous motoneuron activity in FM patients.

Conclusions

The increase in muscle fiber conduction velocity of fibromyalgia patients is independent of the force load and the amount of muscle activity produced, which is indicative of the involvement of the muscle membrane. The increase in membrane conduction velocity probably involves a broad range of muscle fiber types. The muscle fiber conduction velocity of subjects with fibromyalgia may increase excessively when adopting and maintaining a fixed limb position. This suggests that higher regulatory motor centers are involved in this process. Measuring muscle fiber conduction velocity/membrane hyperactivity may bring us closer to understanding fibromyalgia.

Conflict of Interest Statement

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

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References

Banic B, Petersen-Felix S, Andersen OK, Radanov BP, Villiger PM, Arendt-Nielsen L, et al. Evidence for spinal cord hypersensitivity in chronic pain after whiplash injury and in fibromyalgia. Pain 2004; 107:7-15.

Bengtsson A, Henriksson KG, Larsson J. Muscle biopsy in primary fibromyalgia. Light-microscopical and histochemical findings. Scand J Rheumatol 1986; 15:1-6.

Bergmans J. The negative after potential of human muscle fibres. Arch Int Physiol Biochim 1971; 79:187-188.

Blijham PJ, ter Laak HJ, Schelhaas HJ, van Engelen BG, Stegeman DF, Zwarts MJ. Relation between muscle fiber conduction velocity and fiber size in neuromuscular disorders. J Appl Physiol 2006; 100:1837-1841.

Buchthal F, Guld C, Rosenfalck P. Propagation velocity in electrically activated muscle fibres in man. Acta Physiol Scand 1955; 34:75-89. doi: 10.1111/j.1748-1716.1955.tb01227.x

Buchthal F, Sten-Knudsen O. Impulse propagation in striated muscle fibers and the role of the internal currents in activation. Ann N Y Acad Sci 1959; 81:422-445.

Burgmer M, Pfleiderer B, Maihofner C, Gaubitz M, Wessolleck E, Heuft G, et al. Cerebral mechanisms of experimental hyperalgesia in fibromyalgia. Eur J Pain 2012; 16:636-647. doi: 10.1002/j.1532-2149.2011.00058.x

Casale R, Sarzi-Puttini P, Atzeni F, Gazzoni M, Buskila D, Rainoldi A. Central motor control failure in fibromyalgia: a surface electromyography study. BMC Musculoskelet Disord 2009; 10:78.

Choi W, Lim M, Kim JS, Chung CK. Habituation deficit of auditory N100m in patients with fibromyalgia. Eur J Pain 2016; 20:1634-1643. doi: 10.1002/ejp.883

Desmeules JA, Cedraschi C, Rapiti E, Baumgartner E, Finckh A, Cohen P, et al. Neurophysiologic evidence for a central sensitization in patients with fibromyalgia. Arthritis Rheum 2003; 48:1420-1429.

Dunkl PR, Taylor AG, McConnell GG, Alfano AP, Conaway MR. Responsiveness of fibromyalgia clinical trial outcome measures. J Rheumatol 2000; 27:2683-2691.

Elert JE, Rantapaa Dahlqvist SB, Henriksson-Larsen K, Gerdle B. Increased EMG activity during short pauses in patients with primary fibromyalgia. Scand J Rheumatol 1989; 18:321-323.

Ganong WF. Physiology of Nerve & Muscle Cells. In: W.F. Ganong (Ed.), The Nervous System (2nd ed., pp. 21-51). Los Altos, California: Lange Medical Publications; 1979.

Ge HY, Nie H, Madeleine P, Danneskiold-Samsoe B, Graven-Nielsen T, Arendt-Nielsen L. Contribution of the local and referred pain from active myofascial trigger points in fibromyalgia syndrome. Pain 2009; 147:233-240. doi: 10.1016/j.pain.2009.09.019

Ge HY, Wang Y, Danneskiold-Samsoe B, Graven-Nielsen T, Arendt-Nielsen L. The predetermined sites of examination for tender points in fibromyalgia syndrome are frequently associated with myofascial trigger points. J Pain 2010; 11:644-651. doi: 10.1016/j.jpain.2009.10.006

Gerdle B, Ostlund N, Gronlund C, Roeleveld K, Karlsson JS. Firing rate and conduction velocity of single motor units in the trapezius muscle in fibromyalgia patients and healthy controls. J Electromyogr Kinesiol 2008; 18:707-716.

Gracely RH, Petzke F, Wolf JM, Clauw DJ. Functional magnetic resonance imaging evidence of augmented pain processing in fibromyalgia. Arthritis Rheum 2002; 46:1333-1343.

Granit, R. Reconsidering the 'alpha-gamma switch' in cerebellar action. In F. C. Rose (Ed.), Physiological Aspects of Clinical Neurology (pp. 201-214). Oxford: Blackwell Scientific Publications; 1977.

Gydikov A, Christova L. Effect of short interstimulus intervals on the electrically evoked potentials in human muscles. Electromyogr Clin Neurophysiol 1984; 24:137-153.

Henneman E, Somjen G, Carpenter DO. Excitability and inhibitability of motoneurons of different sizes. J Neurophysiol 1965; 28:599-620.

Hogrel JY, Duchene J, Marini JF. Variability of some SEMG parameter estimates with electrode location. J Electromyogr Kinesiol 1998; 8:305-315.

Hubbard DR, Berkoff GM. Myofascial trigger points show spontaneous needle EMG activity. Spine (Phila Pa 1976) 1993; 18:1803-1807.

Hubbard JI, Stenhouse D, Eccles RM. Origin of synaptic noise. Science 1967; 157:330-331.

Islam MS, Larsson O, Nilsson T, Berggren PO. Effects of caffeine on cytoplasmic free Ca2+ concentration in pancreatic beta-cells are mediated by interaction with ATP-sensitive K+ channels and L-type voltage-gated Ca2+ channels but not the ryanodine receptor. Biochem J 1995; 306 (Pt 3): 679-686.

Jacobs JW, Geenen R, van der Heide A, Rasker JJ, Bijlsma JW. Are tender point scores assessed by manual palpation in fibromyalgia reliable? An investigation into the variance of tender point scores. Scand J Rheumatol 1995; 24:243-247.

Jensen KB, Kosek E, Petzke F, Carville S, Fransson P, Marcus H, et al. Evidence of dysfunctional pain inhibition in Fibromyalgia reflected in rACC during provoked pain. Pain 2009; 144:95-100. doi: 10.1016/j.pain.2009.03.018

Kernell D. Basic neuromuscular properties. In: D. Kernell (editor). The Motoneurone and its Muscle Fibres (pp. 5-28). New York: Oxford University Press; 2006.

Klaver-Krol EG. What is known about Electrophysiological Muscle Functions of Patients with Fibromyalgia Syndrome? A review. Fibrom Open Access 2017; 2:125. https://www.omicsonline.org/open-access/what-is-known-about-electrophysiological-muscle-functions-of-patients-withfibromyalgia-syndrome.pdf

Klaver-Krol EG, Henriquez NR, Oosterloo SJ, Klaver P, Bos JM, Zwarts MJ. Distribution of motor unit potential velocities in short static and prolonged dynamic contractions at low forces: use of the within-subject's skewness and standard deviation variables. Eur J Appl Physiol 2007; 101:647-658.

Klaver-Krol EG, Henriquez NR, Oosterloo SJ, Klaver P, Kuipers H, Zwarts MJ. Distribution of motor unit potential velocities in the biceps brachii muscle of sprinters and endurance athletes during short static contractions at low force levels. J Electromyogr Kinesiol 2010; 20:1107-1114. doi: 10.1016/j.jelekin.2010.05.008

Klaver-Krol EG, Zwarts MJ, Ten Klooster PM, Rasker JJ. Abnormal muscle membrane function in fibromyalgia patients and its relationship to the number of tender points. Clin Exp Rheumatol 2012; 30(6 Suppl 74):44-50.

Kosek E, Ekholm J, Hansson P. Sensory dysfunction in fibromyalgia patients with implications for pathogenic mechanisms. Pain 1996; 68:375-383.

Kuan TS, Hsieh YL, Chen SM, Chen JT, Yen WC, Hong CZ. The myofascial trigger point region: correlation between the degree of irritability and the prevalence of endplate noise. Am J Phys Med Rehabil 2007; 86:183-189. doi: 10.1097/PHM.0b013e3180320ea7

Kutner MH. Repeated Measures and Related Designs. In: M. H. Kutner, L. C. Nachtsheim, J. Neter, W. Li (editors). Applied Linear Statistical Models (5th ed., pp. 1157-1161.). New York: McGraw-Hill/Irwin; 2005.

Lange F, Van Weerden TW, Van Der Hoeven JH. A new surface electromyography analysis method to determine spread of muscle fiber conduction velocities. J Appl Physiol 2002; 93:759-764.

Martin A, Carpentier A, Guissard N, van Hoecke, J, Duchateau J. Effect of time of day on force variation in a human muscle. Muscle Nerve 1999; 22:1380-1387.

Mease P. Fibromyalgia syndrome: review of clinical presentation, pathogenesis, outcome measures, and treatment. J Rheumatol 2005; Suppl, 75:6-21.

Merletti R, Rainoldi A, Farina D. Surface electromyography for noninvasive characterization of muscle. Exerc Sport Sci Rev 2001; 29:20-25.

Mottram CJ, Jakobi JM, Semmler JG, Enoka RM. Motor-unit activity differs with load type during a fatiguing contraction. J Neurophysiol 2005; 93:1381-1392.

Simons DG. Do endplate noise and spikes arise from normal motor endplates? Am J Phys Med Rehabil 2001; 80:134-140.

Truini A, Gerardi MC, Di Stefano G, La Cesa S, Iannuccelli C, Pepe A, et al. Hyperexcitability in pain matrices in patients with fibromyalgia. Clin Exp Rheumatol 2015; 33(1 Suppl 88), S68-72.

Truini A, Tinelli E, Gerardi MC, Calistri V, Iannuccelli C, La Cesa S, et al. Abnormal resting state functional connectivity of the periaqueductal grey in patients with fibromyalgia. Clin Exp Rheumatol 2016; 34(2 Suppl 96), S129-133.

Van der Hoeven JH, Lange F. Supernormal muscle fiber conduction velocity during intermittent isometric exercise in human muscle. J Appl Physiol 1994; 77:802-806.

van der Ploeg RJ, Fidler V, Oosterhuis HJ. Hand-held myometry: reference values. J Neurol Neurosurg Psychiatry 1991; 54:244-247.

Vitali C, Tavoni A, Rossi B, Bibolotti E, Giannini C, Puzzuoli L, et al. Evidence of neuromuscular hyperexcitability features in patients with primary fibromyalgia. Clin Exp Rheumatol 1989; 7:385-390.

Wolfe F and Rasker J. Fibromyalgia. In: G. Firestein, R. Budd, S. Gabriel, I. McInnes, J. O'Dell (editors). Kelley's Textbook of Rheumatology (9th ed., pp. 733-752): Elsevier; 2013.

Wolfe F, Simons DG, Fricton J, Bennett RM, Goldenberg DL, Gerwin R, et al. The fibromyalgia and myofascial pain syndromes: a preliminary study of tender points and trigger points in

persons with fibromyalgia, myofascial pain syndrome and no disease. J Rheumatol 1992; 19:944-951.

Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DL, et al. The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. Arthritis Rheum 1990; 33:160-172.

Yunus M, Masi AT, Calabro JJ, Miller KA, Feigenbaum SL. Primary fibromyalgia (fibrositis): clinical study of 50 patients with matched normal controls. Semin Arthritis Rheum 1981; 11:151-171.

Yunus MB. Central sensitivity syndromes: a new paradigm and group nosology for fibromyalgia and overlapping conditions, and the related issue of disease versus illness. Semin Arthritis Rheum 2008; 37:339-352. doi: 10.1016/j.semarthrit.2007.09.003

Table 1. Characteristics of the subjects.

	Patients with fibromyalgia (n = 22)	Controls (n = 21)	Difference P values
Age (years)	44 (10)	44 (15)	0.980
Height (cm)	170.0 (7.5)	169.5 (5.5)	0.749
Body mass (kg)	69.8 (11.1)	65.4 (5.1)	0.107
MVC [a] (Newton)	83.9 (11.4)	90.9 (11.0)	0.047
Duration of complaints (years)	16 (11)	N.A.	N.A.
Self-reported pain [b]	6.2 (1.4)	0.4 (0.8)	< 0.001
Number of tender points [c]	17 (1)	2 (3)	< 0.001
Total TP pain intensity score [d]	27 (8)	3 (3)	< 0.001
Skin thickness (mm) [e]	5.9 (2.3)	5.3 (2.3)	0.405
Skin temperature (° C) [f]	30.9 (0.8)	30.7 (1.1)	0.582

[a] Maximum voluntary contraction force, maximum strength; [b] Experienced pain, reported on the 0 to 10 cm visual analogue scale (VAS, 0 = no pain, 10 = worst pain imaginable); [c] Number of standardized locations at the muscles (tender points, TPs) where the manual palpation was painful (range 0 to 18); [d] Total TP pain intensity score, a sum of pain intensities from 18 standardized locations at the muscles, measured by palpation on a four-point scale (range 0 to 54). [e] Skin thickness on the spot where the electrodes were placed. [f] Temperature near the spot where the electrodes were placed. All subjects are women. Values are mean (SD).

Legends for figures.

Figure 1. Scheme for the selection of the motor unit action potentials' peaks from a surface electromyography signal.

The peak selection has been performed in 200 ms epochs of two differential signals obtained in the length of muscle fibers, for each signal separately. A. A 200 ms epoch. S1, signal 1; S2, signal 2. Choosing the highest motor unit action potential (MUP) in the 200 ms epoch and determining its peak-to-peak amplitude. B. Segment 'A': it is a part of the 200 ms epoch, shown at a slower time base. Finding declines. In each of the two signals, declines are being found with a moving time window. A decline is a diminishing of the signal of at least 20% of the amplitude of the highest motor unit action potential in the 200 ms epoch, over a period of at least 4 s. C. Segment 'A'. Finding peaks in each signal separately. A peak is the highest point prior to a decline (arrows). Finding pairs of peaks between the two signals. A paired peak in the second signal is being searched with a time window of 2 to 6 ms after the peak of the first signal; this corresponds with the expected MUP propagation velocities of 2.5 to 7.5 m/s. D. The peaks found in the first signal, following the definition.

Figure 2. Muscle fiber conduction velocity and the amount of produced electromyographic activity in the muscles of patients with fibromyalgia and healthy controls.

The graph shows the results of two studies: the present study (column 1) and an earlier study (Klaver-Krol et al., 2012) (column 2). From the biceps brachii muscle, two variables were extracted during short static tests: the muscle fiber conduction velocity (CV), and the average rectified voltage (ARV, the amount of muscle activity). FM, patients with fibromyalgia; C,

healthy controls. Force, force level expressed in percentage of maximum voluntary contraction force (MVC). The CV of FM patients is higher than that of the controls. The CV increase in FM patients is unrelated to the force level and the amount of activity produced. There is no difference in the ARV between groups.

Figure 3. Example of illustrative histograms of the motor unit action potential velocities of a female patient with fibromyalgia and a healthy matched control person.

The motor unit action potential propagation velocities (CV-MUPs) are obtained from the biceps brachii muscle during static tests unloaded, and loaded by 5%, 10% and 20% of maximum voluntary contraction force (MVC). Row A: a characteristic patient with fibromyalgia (FM); row B: a healthy control person. Note that the CV-MUPs of the FM patient are globally shifted towards higher values: the lowest CV-MUPs are relatively high (a-arrows), and the highest CV-MUPs are very high (b-arrows).