Later stages of diabetic neuropathy affect the complexity of the neuromuscular system at the knee during low-level isometric contractions

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LATER STAGES OF DIABETIC NEUROPATHY AFFECT THE COMPLEXITY OF THE NEUROMUSCULAR SYSTEM AT THE KNEE DURING LOW-LEVEL ISOMETRIC CONTRACTIONS

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Ethical Publication Statement

“We, the authors, confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.”

Disclosure of Conflicts of Interest

“None of the authors has any conflict of interest to disclose.”

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ABSTRACT

Introduction: The aim of this study was to evaluate the complexity of force and surface electromyography (sEMG) during knee extension and flexion at low-level isometric contractions in individuals with different degrees of diabetic peripheral neuropathy (DPN). Methods: Ten control and 38 diabetic subjects performed isometric contractions at 10%, 20% and 30% of maximal voluntary contraction. Knee force and multichannel sEMG from vastus lateralis (VL) and biceps femoris (BF) were acquired. The standard deviation (SD) of force and sample entropy (SaEn) of both force and sEMG were computed. Results: Subjects with moderate DPN demonstrated high force SD and low force SaEn. Severely affected subjects showed low SaEn in VL at all force levels. Discussion: DPN affects the complexity of the neuromuscular system at the knee for the extension task during low-level isometric contractions with subjects in the later stages of the disease (moderate and severe) demonstrating most of the changes.

KEY WORDS: high-density EMG, motor variability, force, surface electromyography, sample entropy, complexity, diabetes mellitus.
INTRODUCTION

More than 80% of leg amputations in diabetic patients occur after injuries or ulcerations in the foot, making the management of diabetic peripheral neuropathy (DPN) extremely relevant. The most commonly reported symptoms of DPN consist of loss of tactile sensitivity, prickling sensations and pain. As DPN progresses, motor neurons develop loss of neuromuscular junctions and axonal degeneration. Loss of axonal function results in the loss of motor units and impaired axonal sprouting. Axonal sprouting allows reinnervation of denervated muscle fibers of intact motor units in the neighborhood and re-establishment of neuromuscular activity. However, the compensatory effects of axonal sprouting are often limited due to long-term loss of motor end plates. These alterations interfere with muscle strength and endurance while performing tasks of daily living. The literature has shown altered activity in the knee muscles in the presence of DPN, especially in the knee extensors. However, most of these studies demonstrating changes in surface electromyography (sEMG) in relation to DPN are based on single channel recordings.

High density sEMG using a grid of electrodes enables recording of the spatio-temporal muscle activity pattern. At low-level contraction forces, the assessment of the spatio-temporal pattern provides information on the variability in the motor unit recruitment pattern. Recording high density sEMG also enables assessment of the level of complexity of the electromyographic activity over a large area. The complexity of a biological system reflects its ability to adapt and function in a constantly changing environment and this complexity arises from the interaction of structural units and regulatory feedback loops. Consequently, structural or functional alterations due to DPN would affect the interactions between sensory and motor inputs and reduce the adaptive capacity of the sensory-motor system. Therefore, studying the effects of DPN on motor complexity would form the basis of a deeper understanding of the different neuromuscular responses to disease status. Furthermore, examining the complexity of sEMG through the progression of the disease in subjects with DPN may provide insights for better rehabilitation protocols taking into account the temporal effect of the disease on motor control.
Fluctuations of the force and sEMG signal during voluntary isometric contractions have been studied as a simplified model of the physiological mechanism that underlies the control of movement in relation to ageing and disease.\textsuperscript{18,21} Therefore, assessing the complexity of motor control in subjects with DPN during isometric contractions performed at low-level contractions appears sound; especially because DPN mostly affects type I motor units, which are activated in these low-level tasks. The aim of this study was to evaluate the complexity of force and sEMG during knee extension and flexion at low-level isometric contractions in diabetic individuals with different degrees of DPN. We hypothesized that DPN will alter the motor complexity leading to an increased amount of variability and a decreased structure of variability in vastus lateralis and biceps femoris sEMG and in the knee extension and knee flexion force outputs in line with previous studies.\textsuperscript{17,22}

**METHODS**

**Subjects**

Thirty-eight subjects with diabetes mellitus were allocated to four groups: 12 individuals without DPN (absent group), 11 with mild DPN (mild group), 6 with moderate DPN (moderate group) and 9 with severe DPN (severe group). All subjects were type 2 diabetics, except one with type 1 from the severe group. Ten healthy non-diabetic individuals were included as a control group. All participants gave signed informed consent. The study was approved by the local Ethics Committee (protocol number 187/13) and was in conformity with the Declaration of Helsinki.

The inclusion criteria for diabetes participants were age between 40 and 68 years, diagnosed as diabetes type 1 or 2 with or without symptoms of DPN. The inclusion criteria for control subjects were an age between 40 and 68 years and no diagnosis of diabetes. The exclusion criteria were: partial or total limb amputation, symptoms related to vestibulopathy (dizziness, vertigo, unsteadiness), plantar ulceration at evaluation time, severe visual deficits or severe retinopathy, severe nephropathy causing edema or requiring hemodialysis, major vascular complications (venous or arterial ulcers), signs of ischemia demonstrated by an ankle-brachial index below 0.6,\textsuperscript{23} Charcot arthropathy, history of poliomyelitis,
We classified the severity of the DPN (degree score 0-10) by a fuzzy system developed by Watari et al. (2014) using three groups of clinical input variables: (i) tactile sensitivity, (ii) vibratory perception, and (iii) presence of typical DPN symptoms [as per “Michigan Neuropathy Score”]. The DPN scores (x) were grouped as follows: (i) $x \leq 2.0$ absence of DPN; (ii) $2.0 < x < 4.5$ mild DPN; (iii) $4.5 \leq x \leq 7.5$ moderate DPN, and (iv) $x > 7.5$ severe DPN. If ulceration history was present, the individual was automatically classified as severe DPN.

Experimental procedures

**Protocol**

The experimental session was conducted by a physical therapist blinded to the DPN classification and was always conducted in the same order: (i) vastus lateralis muscle (VL) sEMG and knee extension force recordings and (ii) biceps femoris muscle (BF) sEMG and knee flexion force measurement. These muscles were chosen to have one representative of knee flexion and extension that has been described as altered in diabetic patients. Prior to the sEMG and force assessments, the subjects performed familiarization tasks (knee flexion and extension) aiming to achieve the requested force level with a visual feedback system. The assessments were made as follows:

1. Maximal voluntary contraction (MVC) was recorded twice (3s with 1min pause) to determine the subject’s maximal knee force (flexion or extension). The maximal peak force level achieved during both trials was used as the reference for the next recordings.

2. Low-level force recordings of six isometric contractions (10s each, 1min pause) of each task in the following order: Two contractions at 10%MVC, 20%MVC and 30%MVC for knee flexion and extension. Visual feedback was given and an error of ±2% from the target level was accepted. Recordings started after the participant was able to stabilize and maintain the required force level for the specific task. The recordings were performed on the dominant leg in one session.
electrode matrix, the subjects were positioned in a racing car seat adapted in a customized knee dynamometer with the knee in 45° flexion (0°: full extension) and hip flexed at 90°. The trunk was resting in the back of the seat and the seating position was maintained using seat belts (Figure 1).

High-density sEMG and isometric force recordings

High-density sEMG from VL and BF muscles was acquired via matrices of 64 electrodes (ELSCH064NM2 model, OT Bioelettronica, Turin, Italy). The matrix consisted of 13 rows and 5 columns with one missing electrode (2-mm diameter, 8-mm inter-electrode distance in both directions). Prior to attaching the matrix, the skin was shaved, lightly abraded and cleaned to diminish skin-electrode impedance. To determine the matrix location, active contractions were performed against manual resistance by the examiner. The matrix was positioned with its columns along the longitudinal axis of the muscles according to anatomical landmarks: for VL, anterior superior iliac spine and lateral border of patella; for BF, ischial tuberosity and lateral condyle of tibia. The matrices were attached to the skin by means of adhesive foam and the fixation was reinforced with transparent tape. A conductive cream was inserted into each cavity of the foam to assure proper skin-electrode contact. The reference electrode was fixed at the calcaneus tendon in its midway portion. The sEMG signals were recorded monopolarly, amplified 1000 times, sampled at 2048Hz (256-channel sEMG amplifier, EMG-USB2+, OT Bioelettronica, Torino, Italy; -3dB bandwidth 10-500Hz), and digitized with a 12 bit A/D converter. Force signals were amplified 100 times and fed to the auxiliary input present in the EMG-USB2+ and sampled synchronously with the sEMGs.

The knee force was measured with a strain-gage load cell (traction/compression, 1000 N range) customized in a dynamometer (COR1, OT Bioelettronica, Turin, Italy). The knee joint axis of rotation was aligned with the dynamometer axis of rotation. The thigh was firmly strapped to the wooden board connected to the strain gauge transducer.

Force data analysis

The data analysis was performed offline with MATLAB R2016a software (MathWorks, Inc.),
Massachusetts, USA). Force data were downsampled to 100Hz and low-pass filtered at 10Hz (4th order). For MVC trials, the mean force was computed over 500ms windows with 100ms overlap and the highest value was considered as the peak force.

For force recordings at 10-20-30%MVC, the amount of force variability was quantified by the standard deviation (SD) of the exerted force over the 5s central epoch of the 10s trial. The force signal was inspected and if the mean force in the selected 5s epoch was not in the ±2% range, a new 5s window was selected for analysis. The selected window was maintained for all force and sEMG analysis. Nonlinear analysis was also performed to assess the structure of force variability. The sample entropy (SaEn) of the exerted force was calculated over the same 5s window as described above. SaEn is the negative natural logarithm of the conditional probability that a set of data with a length N having repeated itself within a tolerance r for m points will also repeat itself for m+1 points. The choice of the embedding dimension (m) and the tolerance distance (r) values used to respectively define the space state and similarity criterion are crucial. We set m to 2 and r to 0.2xSD of the force signal in line with previous studies. SaEn is a unitless, non-negative number with higher values indicating a less regular, unpredictable and complex structure of the signal time series.

High-density sEMG data analysis

The recorded VL and BF monopolar sEMG signals were off-line band-pass filtered (10-500 Hz, 4th order Butterworth filter). A notch filter of 60Hz and its harmonics (120Hz, 180Hz, 240Hz, 300Hz, 360Hz, 420Hz and 480Hz) were applied to diminish the interference of the power line. Fifty-nine bipolar sEMGs were obtained along the columns of electrodes (12x5 bipolar recordings with one missing electrode) considering the longitudinal axis of the muscle. At each force level, root mean square (RMS) values of the bipolar sEMG were computed over the same epoch used to compute the force level divided in five consecutive 1s epochs and averaged; thus providing RMS maps for each performed task. For MVC contractions, the RMS map was obtained for the epoch corresponding to the abovementioned maximum contraction. Further, the mean value of the RMS map from MVC was obtained and set as a reference value.
The mean RMS value from each map was normalized by the reference value obtained from the MVC. The normalized values represented the level activation of the studied muscle.

SaEn was also computed for sEMG data \((m=2\text{ and } r=0.2xSD)\) to characterize the complexity of the sEMG. SaEn was calculated over the same five consecutive 1s epochs without overlap for each bipolar derivation. The mean of SaEn obtained from five epochs was calculated and a SaEn map was obtained. The mean value from the SaEn map was considered as the measure of the complexity level. Thus, RMS and SaEn values represent two different constructs of the neuromuscular knee control.32

**Statistical analysis**

Descriptive statistics are reported as mean (SD). Demographics, anthropometrics and clinical variables were compared among groups using analyses of variance (ANOVAs), followed by Bonferroni post-hoc test or Chi-square tests for categorical data. Normality of all variables was confirmed with Shapiro-Wilks tests \((P>0.05)\). Multivariate analysis of variance (MANOVA) was performed for the knee flexion and knee extension force variables (SD force, SaEn force) at all force levels (10-20-30%MVC) with the independent factor containing five levels (control, absent, mild, moderate and severe). When a significant multivariate effect was observed, univariate ANOVAs were performed with Bonferroni post-hoc tests corrected for pairwise comparisons. The same analysis was repeated for VL and for BF sEMG variables (RMS, SaEn sEMG). Effect sizes and their confidence intervals (CIs) with 95% confidence were estimated with Hedges’s \((g)\) for group comparisons and \(g\) values were considered small between 0.2 and 0.5, medium between 0.5 and 0.8, and large above 0.8.33 The significance level was set at 5%, but since our study maybe under-powered due to the small sample size, we have indicated the level of the effect size regardless of finding a significant effect. All statistical analyses were performed with IBM SPSS Statistics 23.0 (IBM, New York, USA).

**RESULTS**

Table 1 shows the demographic, anthropometric and clinical information of the participants.

Mean force, amount and structure of force variability during knee extension
No statistical differences were found in the mean of the peak force values among groups for knee extension (control = 325.7 ± 141.5 N; absent = 294.7 ± 86.2 N; mild = 295.1 ± 108.7 N; moderate = 371.0 ± 112.1 N; severe = 335.8 ± 118.3 N; F=0.585, P=0.675).

The ANOVA results, Hedge’s effect sizes and their corresponding confidence intervals are reported in the supplementary material (Supplementary Table S1). The MANOVA output showed a significant difference in the knee extension force during low-level contractions among groups (Wilk’s Λ=0.500, F=2.756, P<0.001). The ANOVA detected a significant group effect on force SD at 10 (F=6.382, P<0.001), 20 (F=3.878, P=0.006) and 30% of MVC tasks (F=5.392, P=0.001). Figure 2 shows the mean (±SD), post-hoc comparisons and effect sizes of the SD and SaEn during knee extension. At 10%MVC, mild DPN demonstrated a significantly higher SD force compared with the control group and moderate DPN demonstrated a significantly higher SD force compared with the control and absent groups. At 20%MVC, the moderate DPN had significantly higher force SD than the control group and at 30%MVC the moderate DPN had higher force SD than controls, absents and mild DPN. The ANOVA also revealed a significant group effect on force SaEn at 10% (F=4.890, P=0.001) and 20%MVC (F=3.653, P=0.008). See Figure 2 for the results of the post-hoc comparisons and effect sizes. At 10%MVC, the moderate DPN had lower force SaEn than controls and absent and the severe DPN presented lower force SaEn than controls. At 20%MVC, absent and moderate groups showed significantly lower SaEn values than controls.

**Mean force, amount and structure of force variability during knee flexion**

No statistical differences were found in the mean of the peak force values among groups for the knee flexion (control = 212.9 ± 119.3 N; absent = 160.5 ± 61.6 N; mild = 152.2 ± 85.4 N; moderate = 166.5 ± 83.9 N; severe = 156.2 ± 87.1 N; F=0.765, P=0.554).

The ANOVA results, the Hedge’s effect sizes and their corresponding confidence intervals are reported in the supplementary material (Supplementary Table S2). The MANOVA output did not show a significant difference in knee flexion force during low-level contractions among groups (Wilk’s Λ=0.689, F=1.334, P<0.140). However, the effect sizes showed that severe individuals presented higher force SaEn
than controls at 10%MVC and higher force SaEn than controls, mild and moderate groups at 20% MVC (large effect sizes). Figure 3 shows the mean (±SD) and effect sizes of the SD and SaEn during knee flexion.

Figure 4 shows the mean (±SD), post-hoc comparisons and effect sizes of the RMS and SaEn of VL during knee extension. The ANOVA results, Hedge’s effect sizes and their corresponding confidence intervals are reported in the supplementary material (Supplementary Table S3). MANOVA output showed significant differences of sEMG variables among groups (Wilk’s Λ=0.498, F=2.774, P<0.001) and univariate ANOVAs showed a significant group effect for VL SaEn at 10% (F=4.94, P=0.001), 20% (F=2.79, P=0.031) and 30% of MVC (F=2.65, P=0.038). At 10% MVC, post-hoc comparisons showed that the neuropathic groups (mild, moderate and severe) had lower SaEn than the control group. At 20% and 30%MVC, the severe subjects presented significantly lower SaEn when compared with controls.

Amplitude, amount and structure of sEMG variability during knee flexion

Figure 5 shows the mean (±SD), post-hoc comparisons and effect sizes of the RMS and SaEn of the BF during knee flexion. The ANOVA results, Hedge’s effect sizes and their corresponding confidence intervals are reported in the supplementary material (Supplementary Table S4). MANOVA output showed significant differences of sEMG variables among groups (Wilk’s Λ=0.609, F=1.806, P=0.013) and the univariate ANOVAs showed a significant group effect for BF SaEn at 10%MVC (F=2.977, P=0.024). Post-hoc comparisons showed that severe subjects had significantly lower BF SaEn than controls at 10%MVC.

DISCUSSION

As hypothesized, we found an increased amount and a lower structure of force variability in DPN individuals during knee extension, mainly at 10%MVC, and a lower VL sEMG complexity in severe diabetics at all contraction levels.

All participants were able to maintain the required force levels for 10s. An optimal amount of variability in a biological system has been proposed to be directly associated with health. The structure of the variability, characterized in this study by computing SaEn, is also an important aspect since a lower complexity of a time series (i.e. with lower structure) has been associated with disease.
During knee extension at 10%MVC, mild and moderate DPN subjects demonstrated higher force SD than healthy subjects, and moderate and severe DPN subjects had both higher force SD and lower force SaEn than controls and subjects without DPN. During knee extension at 20%MVC, moderate and absent DPN subjects had higher force SD and lower force SaEn than controls. At 30%MVC moderate DPN subjects showed higher force SD than controls, mild and absent DPN subjects. The presence of DPN, mainly starting from a moderate degree, affects the amount and structure of variability of force delineating a less adaptable system.\(^\text{35}\) The amount of torque variability during isometric contractions has been shown to be correlated with motor performance; with larger plantar flexor torque variability being associated with e.g. a less stable balance.\(^\text{36,37}\) Further, the practice of isometric plantar flexion contractions within 20% of MVC has been shown to diminish the amount of plantar flexion torque variability and improve the postural stability.\(^\text{38}\)

Although we cannot directly infer that the greater amount of variability in knee extensor torque would affect motor control during the performance of daily tasks, the changes in variability of the force observed in the DPN subjects might be detrimental during the performance of daily tasks since most daily activities, such as walking, squatting and climbing stairs, on average require between 10% and 30% of MVC activity for quadriceps and hamstring muscles.\(^\text{39}\) Enhancing the motor control during the performance of isometric knee contractions via knee strengthening programs will probably benefit DPN patients and may be taken into consideration during rehabilitation.

Parallel to the changes observed in the force variability, DPN diminished the complexity in VL sEMG particularly at 10%MVC while no major differences were found in the sEMG amplitude. All DPN groups (i.e. mild, moderate and severe) showed lower VL SaEn when compared with the matched control group as stated by Golderberg et al\(^\text{18}\). Further, at 20 and 30%MVC only severe subjects showed lower SaEn compared with controls. The structure of variability depicts how the system output changes over time.\(^\text{40}\) Thus, the lower structure of variability in sEMG represents a less complex and unpredictable system characterized by a reduced functional capacity.\(^\text{41}\) Further, the low SaEn values of VL activity in severe DPN
subjects most likely depict a lower ability to take benefit of the redundancy of the motor system. With the progression of DPN, the motor neurons can present signs of neuromuscular junction loss and axonal degeneration which result in loss of motor units characterizing a loss of functional components in the motor system that would lead to the observed low complexity in severe patients. Watanabe et al. demonstrated that individuals with type-2 diabetes mellitus have a limited area of activation within VL muscles with an attenuated spatial pattern of sEMG compared with age-matched control subjects. The sEMG was measured during isometric knee extension at 10%MVC with a 64-channel matrix and the spatial distribution was expressed as the modified entropy from the RMS values. The authors interpreted this finding as indicating a limited number of motor units recruited to perform knee extension. The VL muscle has an important function during locomotor activities since it eccentrically controls the knee flexion during the loading phase. Alterations in intra-limb joint coordination during gait have been described in DPN subjects, and a less variable pattern is observed in DPN subjects in comparison with healthy subjects. This lower variability is observed in the ankle-knee and knee-hip continuous relative phase during the early stance phase when the knee contributes eccentrically to the load attenuation and in the knee-hip relative phase during the terminal swing phase when the knee assumes the important task of propelling the limb. Therefore, special attention should be addressed to the knee extensor muscles and synergistic muscle actions in the rehabilitation of DPN patients.

The amount of variability during knee flexion did not show a significant difference among groups. Differences in the structure of force variability were only observed in the effect sizes at 10%MVC where the severe group presented a higher SaEn than the control group and at 20%MVC where the severe group presented a higher SaEn than the control, mild and moderate groups. Such inverse relationships have been reported earlier and can be explained by taking in consideration complexity tradeoffs between macroscopic (force or movement) and microscopic (sEMG) levels of a system. For the BF the only significant difference was observed for SaEn at 10%MVC with the severe group showing lower complexity than controls. It can be argued that the isometric contraction performed during the knee flexion task...
performed in the studied setup (sitting position and knee flexion at 45°) did not recruit enough motor units allowing detection of differences between the studied groups. As such, the developed forces were low, corresponding to approximately half of the values presented during knee extension most likely due to the fact that the participants reported difficulty to perform MVC knee flexions and discomfort. Another limitation of the study was the small sample size of the moderate and severe groups that could have masked the existence of additional alterations due to DPN; apart from what is described above.

Overall, the changes demonstrated in force and sEMG variability during knee extension showed that DPN in the later stages of the disease most likely plays a major role in the observed alterations in the motor control underlying the clinical findings in these patients. Subjects with moderate DPN showed a consistent pattern of alterations since they presented a higher amount of force variability in all the studied force levels while both moderate and severe subjects presented a lower structure of force variability. Severe subjects also showed a lower structure of sEMG in the VL at all studied force levels. DPN is a progressive disease and therefore the presence of alterations in the later stages of the disease was expected. Further, the fact that most of the changes were observed at 10%MVC suggests the relevance of studying low-level contractions as the motor system has more latitude to adapt motor strategies. The force signal is influenced by the discharge variability of the active motor units and DPN probably interferes with the recruitment of motor units and firing characteristics. Additionally, the fact that the subjects with moderate DPN showed consistent findings might reflect that some of the more severe subjects show some level of recovery of the motor control. Axonal sprouting is a response to the denervation caused by DPN resulting in reinnervation of the muscle fibers by the intact motor units from the neighborhood. The compensatory effects of the axonal sprouting are considered limited, but they might have contributed to some extent to the apparent recovery presented in some of the variables in the severe DPN individuals. Co-activation strategies might also have been affected by the disease and its progression and interfere with motor control. Thus, future studies should also compute indexes as co-contraction or mutual information among muscle pairs in relation to DPN. Also, the coordination of synergistic muscles might be affected
since there is evidence that synergic thigh muscles share synaptic input.\textsuperscript{50} Besides that, fibers Ia may be dysfunctional in diabetic patients\textsuperscript{51}. Future studies recording sEMG activity from synergic muscles simultaneously and investigating the effects of DPN on coordination are warranted.

In conclusion, the findings revealed that DPN affects the complexity of the neuromuscular system at the knee for the extension task during low-level isometric contractions. The neuropathic subjects with moderate and severe neuropathy presented the majority of the alterations showing the effects of DPN in the knee motor control. Our results give insights for rehabilitation programs that should focus on restoring neuromuscular control of the knee using, for example, exercise therapy, strength or Taiji training.\textsuperscript{5,32,52,53}

Therefore, rehabilitation protocols for DPN could include tasks that challenge the knee motion control and create new biomechanical solutions. Although most of the alterations occurred at moderate and severe stages, alterations in force and sEMG complexity were present already with mild DPNs, especially at 10\%MVC. Thus, interventions should also be planned in the early stages of DPN.
List of abbreviations

ANOVA = analyses of variance
BF = biceps femoris
CIs = confidence intervals
DPN = diabetic peripheral neuropathy
MANOVA = multivariate analysis of variance
MVC = maximal voluntary contraction
RMS = root mean square values
SaEn = sample entropy
SD = standard deviation
sEMG = surface electromyography
VL = vastus lateralis
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Figure Legends

Figure 1 – Experimental setup illustrating the electrode matrix placed at vastus lateralis for sEMG recordings with the knee positioned at 45º degrees (0º = full extension) in the dynamometer.

Figure 2 – Mean (±SD) of the standard deviation (SD, N) and sample entropy (SaEn, unitless) of the knee extension force at 10%, 20% and 30% of MVC. Lines and circles represent medium and large effect sizes with dotted lines representing medium effect sizes, solid lines representing large effect sizes, and circles representing the presence of large or medium effect sizes for all pairwise estimates. * Statistical differences detected in post-hoc tests.

Figure 3 – Mean (±SD) of the standard deviation (SD, N) and sample entropy (SaEn, unit less) of the knee flexion force at 10%, 20% and 30% of MVC. Lines and circles represent medium and large effect sizes with dotted lines representing medium effect sizes, solid lines representing large effect sizes, and circles representing the presence of large or medium effect sizes for all pairwise estimates.

Figure 4 – Mean (±SD) of root mean square (RMS, %MVC) and sample entropy (SaEn, unit less) of vastus lateralis muscle at 10, 20 and 30% of MVC. Lines and circles represent medium and large effect sizes with dotted lines representing medium effect sizes, solid lines representing large effect sizes, and circles representing the presence of large or medium effect sizes for all pairwise estimates. * Statistical differences detected in post-hoc tests.

Figure 5 – Mean (±SD) of root mean square (RMS, %MVC) and sample entropy (SaEn, unit less) of biceps femoris muscle at 10, 20 and 30% of MVC. Lines and circles represent medium and large effect sizes with dotted lines representing medium effect sizes, solid lines representing large effect sizes, and circles representing the presence of large or medium effect sizes for all pairwise estimates. * Statistical differences detected in post-hoc tests.
Table 1 – Anthropometric variables and clinical data (mean ± standard deviation) for the experimental groups.

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<td>0.63</td>
<td>1.0 ± 0.6</td>
<td>2.9 ± 0.9²</td>
<td>5.1 ± 0.5³</td>
<td>7.6 ± 1.9²</td>
<td>&lt;0.001³</td>
</tr>
</tbody>
</table>

*ANOVA test, ‡Chi-square test. #Group statistically different from all others – Bonferroni pairwise comparisons.
Figure 1 – Experimental setup illustrating the electrode matrix placed at vastus lateralis for sEMG recordings with the knee positioned at 45° degrees (0° = full extension) in the dynamometer.

FIGURE 1 near here
192x144mm (300 x 300 DPI)
Figure 2 – Mean (±SD) of the standard deviation (SD, N) and sample entropy (SaEn, unit less) of the knee extension force at 10%, 20% and 30% of MVC. Lines and circles represent medium and large effect sizes with dotted lines representing medium effect sizes, full lines representing large effect sizes, and circles representing the presence of large or medium effect sizes for all pairwise estimates. * Statistical differences detected in post-hoc tests.

FIGURE 2 near here
219x174mm (240 x 240 DPI)
Figure 3 – Mean (±SD) of the standard deviation (SD, N) and sample entropy (SaEn, unit less) of the knee flexion force at 10%, 20% and 30% of MVC. Lines and circles represent medium and large effect sizes with dotted lines representing medium effect sizes, full lines representing large effect sizes, and circles representing the presence of large or medium effect sizes for all pairwise estimates.

FIGURE 3 near here

218x175mm (240 x 240 DPI)
Figure 4 – Mean (±SD) of root mean square (RMS, %MVC) and sample entropy (SaEn, unit less) of vastus lateralis muscle at 10, 20 and 30% of MVC. Lines and circles represent medium and large effect sizes with dotted lines representing medium effect sizes, full lines representing large effect sizes, and circles representing the presence of large or medium effect sizes for all pairwise estimates. * Statistical differences detected in post-hoc tests.

FIGURE 4 near here

211x177mm (240 x 240 DPI)
Figure 5 – Mean (±SD) of root mean square (RMS, %MVC) and sample entropy (SaEn, unit less) of biceps femoris muscle at 10, 20 and 30% of MVC. Lines and circles represent medium and large effect sizes with dotted lines representing medium effect sizes, full lines representing large effect sizes, and circles representing the presence of large or medium effect sizes for all pairwise estimates. * Statistical differences detected in post-hoc tests.