ENEIDA YURI SUDA

**Padrões dinâmicos de recrutamento muscular por EMG de alta
densidade de diabéticos em diferentes níveis de severidade de
neuropatia classificados por um modelo linguístico *fuzzy***

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Orientadora: Profa. Dra. Isabel de Camargo
Neves Sacco

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“A ciência é o melhor instrumento para medir nossa ignorância”

Paolo Mantegazza

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RESUMO

Suda EY. *Padrões dinâmicos de recrutamento muscular por EMG de alta densidade de diabéticos em diferentes níveis de severidade de neuropatia classificados por um modelo linguístico fuzzy* [tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2017.

Introdução: O objetivo desse estudo é obter um melhor entendimento de como o controle motor é afetado em indivíduos com polineuropatia diabética (PND). A oscilação da força e da atividade eletromiográfica (EMG) durante contrações voluntárias isométricas têm sido estudados como um modelo simplificado dos mecanismos fisiológicos que estão por trás do controle do movimento. A análise dos padrões de recrutamento muscular dos membros inferiores obtidos pela EMG de alta densidade, juntamente com a análise dos dados de força relativos às articulações do joelho e do tornozelo, sob a ótica da análise da complexidade, contribui para o entendimento dos efeitos da doença no controle dos movimentos. Além disso, analisar a complexidade da força e da atividade muscular de indivíduos diabéticos com PND levando em conta o progresso da severidade da doença, desde os estágios subclínicos aos mais avançados, irá ajudar no entendimento de como as alterações neuromusculares vão se instalando no curso da doença. As hipóteses desse estudo foram: a PND altera a complexidade motora o que se reflete em um aumento na magnitude da variabilidade e em uma diminuição da estrutura da variabilidade nos dados de força e EMG; e essas alterações pioram com a severidade da doença.

Métodos: Foram avaliados 10 sujeitos controles e 38 sujeitos diabéticos, classificados como ausentes, leves, moderados ou graves, em relação ao nível da PND, por um sistema de tomada de decisão *fuzzy* cujas variáveis de entrada são clínicas. Foi adquirido o sinal EMG de alta densidade (matriz de 64 eletrodos) dos músculos tibial anterior, gastrocnêmio medial, vasto lateral e bíceps femoral durante a contração isométrica a 10%, 20% e 30% da contração voluntária máxima. Simultaneamente, foi adquirido o sinal da força isométrica durante a flexão e extensão de joelho e tornozelo. Foram calculados o desvio padrão e a *sample entropy* da força, e o *root mean square* e a *sample entropy* do EMG. As diferenças entre os grupos para as variáveis obtidas da força e do EMG foram verificadas através de análises multivariadas ($\alpha=0,05$).

Resultados: No geral, durante a flexão de tornozelo, sujeitos moderados e graves apresentaram um maior desvio padrão da força, e sujeitos moderados apresentaram menor *sample entropy* da força. Durante a extensão de tornozelo, os sujeitos moderados apresentaram maior desvio padrão da força e todos os grupos diabéticos apresentaram menor *sample entropy* da força. Durante a extensão do joelho, os sujeitos moderados apresentaram um maior desvio padrão e uma menor *sample entropy* da força. O tibial anterior apresentou um maior *root mean square* no grupo ausente e uma menor *sample entropy* nos sujeitos leves. O gastrocnêmio medial

apresentou maior *sample entropy* nos sujeitos moderados e graves. Os sujeitos graves apresentaram menor *sample entropy* no vasto lateral em todos os níveis de força estudados. **Conclusões:** A PND afeta a complexidade do sistema neuromuscular durante contrações isométricas de baixa intensidade, reduzindo a capacidade do sistema de se adaptar às demandas mecânicas desafiadoras. Os padrões de complexidade neuromuscular observados não estavam associados à gravidade da doença, com a maioria das alterações sendo observadas no grupo moderado.

Descritores: neuropatias diabéticas; complexidade; eletromiografia; força muscular; *sample entropy*; lógica fuzzy.

ABSTRACT

Suda EY. *Dynamic patterns of muscular recruitment obtained by high-density EMG from diabetic subjects with different neuropathy degrees classified by a fuzzy-linguistic model* [thesis]. São Paulo: “Faculdade de Medicina, Universidade de São Paulo”; 2017.

Introduction: The general proposal of this study is to get a better understanding on how motor control is affected in diabetic polyneuropathy (DPN) individuals. Fluctuations of the force and sEMG signal during isometric voluntary contractions have been studied as a simplified model of the physiological mechanism that underlies the control of movement. The analysis of the muscular recruitment patterns from lower limbs, obtained by high-density sEMG, together with the corresponding force signal outputs from knee and ankle joints, in the light of complexity analysis, adds to the knowledge of the disease effects in motor control. Additionally, examining the complexity of force and muscle activity of DPN individuals, along with the development of DPN from subclinical to the more severe stages, will help us understanding how the alterations will develop with the DPN progression. The hypotheses of this study are that: DPN alters the motor complexity, expressed as an increased amount of variability and a decreased structure of variability in sEMG and force outputs, and these alterations are aggravated by the severity of the disease.

Methods: We assessed 10 control subjects and 38 diabetic subjects, classified as absent, mild, moderate, or severe DPN, by a fuzzy-system based on clinical variables. Multichannel sEMG (64-electrode matrix) of tibialis anterior, gastrocnemius medialis, vastus lateralis and biceps femoris muscles were acquired during isometric contractions at 10%, 20%, and 30% of the maximum voluntary contraction, and force levels were recorded during dorsiflexion, plantarflexion, knee extension and knee flexion. Standard deviation and sample entropy of force signals were calculated and root mean square and sample entropy were calculated from sEMG signals. Differences among groups of force and sEMG variables were verified using a multivariate analysis of variance ($\alpha=0.05$). **Results:** Overall, during dorsiflexion contractions, moderate and severe subjects had higher force standard deviation and moderate subjects had lower force sample entropy. During plantarflexion, moderate subjects had higher force standard deviation and all diabetic subjects had lower entropy. During knee extension moderate DPN subjects demonstrated high force standard deviation and low force sample entropy. Tibialis anterior presented higher root mean square in absent group and lower entropy in mild subjects. For gastrocnemius medialis, entropy was higher in severe and lower in moderate subjects. Severely affected participants showed low sample entropy in vastus lateralis at all force levels. **Conclusions:** DPN affects the complexity of the neuromuscular system during low-level isometric contractions, reducing the system's capacity to adapt to challenging mechanical demands. The

observed patterns of neuromuscular complexity were not associated with disease severity, with the majority of alterations recorded in moderate subject.

Descriptors: diabetic neuropathies; complexity; electromyography; muscle strength; sample entropy; fuzzy logic.

1. INTRODUCTION

1.1. Diabetic peripheral polyneuropathy and its neuromuscular alterations

Diabetes mellitus is defined as a group of metabolic diseases characterized by the presence of hyperglycemia, which can be caused by a deficiency in the insulin secretion, a deficiency in the insulin action, or by a combination of both conditions (Expert Committee, 2003). The last estimative of the International Diabetes Federation, in 2014, shows that around 387 million people have diabetes in the world, corresponding to 8.3% of the worldwide population. The prevalence in Brazil follows the world trend since 8.68% of the Brazilian adult population have diabetes, corresponding to more than 11 million people (Mena, Sea and Lucia, 2014). Projections are that in the year of 2035 around 592 million people between 20 and 79 years old will have diabetes (Guariguata *et al.*, 2014), corresponding to a 53% increase in the number of cases.

The diabetic peripheral polyneuropathy (DPN) is a common complication in diabetic patients that can affect around 50% of the diabetic population (Tesfaye *et al.*, 2010). DPN affects both sensitive and motor fibers of the limbs, with distal to proximal progression (England *et al.*, 2005; Callaghan *et al.*, 2012). The most commonly reported symptoms consist of loss of tactile sensitivity, prickling sensation and pain, but over 50% of DPN patients might be asymptomatic, presenting a potential risk for foot lesions (Boulton *et al.*, 2005). Although the complications in the diabetic foot

develop from the reciprocal action of a set of factors, DPN seems to be the most important one (Urbancic-Rovan, 2005), being associated to a high risk of foot ulcers and lower limb amputations (Veves *et al.*, 1992; Malyar *et al.*, 2016).

Although the first symptoms of DPN are sensorial, with sensory neurons showing marked structural damages, such as loss of innervation of the skin and retraction of the distal branches, earlier than motor neurons (Zochodne, Ramji and Toth, 2008); some studies have provided evidence for alterations also in motor neurons since early stages of the disease (Meijer *et al.*, 2008; Sung *et al.*, 2012). Thereby, as DPN progresses, motor dysfunction develops, interfering with the neuromuscular activity and the performance of daily living tasks.

Motor dysfunction in DPN is a consequence of both motor neuron impairments and alterations in the properties of the skeletal muscles. Alterations in the motor unit (MU) potentials (Allen *et al.*, 2015), decreased MU firing rates (Allen *et al.*, 2014a), reductions in the motor nerve conduction velocity (Kitano *et al.*, 2004; Ijzerman *et al.*, 2011; Sung *et al.*, 2012), which all reflect the presence of axonal dysfunction, and loss of MU and motor axons (Allen *et al.*, 2013, 2014a, 2015) have been described in DPN subjects. Signs of collateral reinnervation and axonal sprouting have also been described in DPN patients, but it seems that the reinnervation process is unable to keep pace with the rate of axonal loss (Bril *et al.*, 1996; Andersen *et al.*, 1998) and therefore, functional alterations are regularly observed in moderate and severe stages of the disease.

The main alterations in the properties of the skeletal muscles due to DPN are loss of muscle mass (Andersen *et al.*, 1997; Andersen, Gjerstad and Jakobsen, 2004;

Allen *et al.*, 2014b; Moore *et al.*, 2015) and deterioration of contractile quality, defined as the amount of activated muscle to generate force (Ijzerman *et al.*, 2011; Allen *et al.*, 2014b; Moore *et al.*, 2015), both contributing to the described strength loss in this population, especially at the ankle joint (Andersen *et al.*, 1997; Ijzerman *et al.*, 2011; Allen *et al.*, 2013; Allen *et al.*, 2014b).

Additionally, diabetes itself also interferes with the properties of the skeletal muscles. Loss of muscle mass (Guerrero *et al.*, 2016), deterioration of muscle contractile quality (Park *et al.*, 2006; Ijzerman *et al.*, 2011; Guerrero *et al.*, 2016) and strength losses (Park *et al.*, 2006; Ijzerman *et al.*, 2011; Ijzerman *et al.*, 2012) have also been described in diabetic patients without DPN, as well as increases in the muscular lipid content (He, Watkins and Kelley, 2001), impairments in the mitochondrial respiration (Mogensen *et al.*, 2007; Larsen *et al.*, 2009) and decreases in the muscular oxidative activity (He, Watkins and Kelley, 2001). Besides that, a series of biopsy studies in the vastus lateralis muscle of diabetic patients have shown the presence of a greater content of type II muscular fibers (Marin *et al.*, 1994; Gaster *et al.*, 2001; Oberbach *et al.*, 2006; Mogensen *et al.*, 2007; Andersen *et al.*, 2009), suggesting that diabetes affects more the oxidative fibers (type I). Altogether, these alterations interfere in the motor control and, therefore, a better understanding of the motor-control strategies adopted in the presence of DPN will be beneficial for the management of this co-morbidity.

1.2. The diabetic peripheral polyneuropathy progression and the Fuzzy Sets Theory

The assessment of DPN is clinical and involves the identification of specific signs and symptoms, sensorial tests that evaluate neurological impairments and physical examination of the feet (England *et al.*, 2005; Forouzandeh *et al.*, 2005; Asad *et al.*, 2010; Dyck *et al.*, 2011). The gold standard for DPN diagnosis is electroneuromyography since it provides objective information about nerve conduction impairments (Tsfaye *et al.*, 2010; Dyck *et al.*, 2011).

Although electrodiagnostic exams are uncomfortable, invasive, less accessible due to their high costs and need highly specialized professionals and equipment to be properly performed, the criteria for determining the presence of DPN is the detection of abnormalities in any parameter of nerve conduction in two different nerves, being mandatory that the sural nerve is one of them (England *et al.*, 2005). More recently, Dick *et al.* (2011) reported that the most sensitive elements to DPN are the motor nerve conduction velocities of tibial, fibular and ulnar nerves, the action potential of sural nerve, and the F-wave latency of tibial and ulnar nerves.

DPN symptoms as tingling, burning, numbness and prickling, between others, are usually evaluated through validated questionnaires (Boulton *et al.*, 2004). The somatosensorial modalities of assessment recommended by the International Consensus of Diabetic Foot for DPN detection are the testing of pressure sensation under the plantar surface with 10-g Semmes-Weinstein monofilaments, and the testing of vibratory perception with a 128-Hz tuning fork or a biothesiometer (Bakker,

Apelqvist and Schaper, 2012). The severity of DPN has been determined through the combination of neurophysiological alterations, the presence of signs and symptoms and dysfunction scores (Moghtaderi, Bakhshipour and Rashidi, 2006; Tesfaye *et al.*, 2010; Dyck *et al.*, 2011).

DPN has also an insidious manifestation, with the symptoms being monophasic or fluctuating over time (Teskaye *et al.*, 2010), making it difficult to identify precisely its onset for further management. As an example, nerve conduction abnormalities might be present in diabetic patients without signs or symptoms of DPN (Dyck *et al.*, 2011) showing that DPN can be subclinical in the initial stages, and it gets worse along the years, showing a continuous progression that is non-linear (Morimoto *et al.*, 2012). Therefore, patients cannot be simply described as having or not having DPN.

Even though some tools are available to classify DPN severity (Feldman *et al.*, 1994; Lunetta *et al.*, 1998; Meijer *et al.*, 2002; Gin, Perlemoine and Rigalleau, 2006; Leese *et al.*, 2006), the disease has a heterogeneous manifestation, making it difficult to determine what the pool of DPN-related variables mean when different associations are made between them. Without objective criteria to interpret these signs and symptoms associations, conclusions rely on subjective opinions of experts or in systems that use scores that rests on simple sums of results, without considering the weight of each different variable in the diagnosing criteria.

Since there is no consensus about the best way to classify DPN patients, different studies used different ways to define the presence of DPN in the evaluated subjects. Previous biomechanics studies might have contaminated the experimental groups, mixing diabetic patients with and without DPN in the same experimental

group, and grouping subjects with different degrees of neuronal impairment, limiting the interpretation of the results.

In this scenario of an insidious onset and a progressive development of DPN, in which the boundaries between the absence and the presence of this disease is unclear, a method that takes into account the uncertainties of the clinical assessment and that is capable of objectively measure a subjective judgment is extremely useful (Massad *et al.*, 2003; Licata, 2007; Picon *et al.*, 2012).

The binary logic deals with “false” or “true” concepts. Based in this logic, one can classify an individual as “having a disease” or “not having a disease”, for example, and include him/her in a determined set - in this example, in the “healthy set”, or in the “sick set”. The fuzzy logic was introduced by Lofti Zadeh in 1965 in order to deal with the uncertainty in the identification of an element as part or not of a determined set (Zadeh, 1965). The Fuzzy Sets Theory works with the concept of “partial truth”. Within this concept it is possible to grade statements between dichotomous definitions, building ratings between “completely truth” and “completely false” (Massad *et al.*, 2003). The fuzzy set is a “class” with a continuum of grades of membership (Zadeh, 1965) and the fuzzy logic allows the use of a region of concomitance or contradiction of concepts, in which an element can be part of more than one set at the same time, making it possible to combine different criteria to reach conclusions about the disease status with higher precision (Licata, 2007). This method allows classifications between 0-1, i.e., making it possible to allocate individuals at any point between totally healthy (0) and totally sick (1), without crispy boundaries.

This logic was built to deal with uncertainties of a membership class, being an interesting approach to evaluate the degree of DPN, since a clear identification of the onset of this disease and its development is fuzzy and not crispy. The logical structure of the Fuzzy Sets Theory allows the use of linguistic variables, that are subjective, to classify the measured variable in a study (DPN severity, for instance), providing a numerical value as an output, that corresponds to the degree in which an element is a member of a determined fuzzy set (Massad *et al.*, 2003).

Duckstein *et al.* (1995) developed a diagnosis system for DPN based on the fuzzy logic using nerve conduction studies as the input variable to this classification. The system allowed creating four classifications for DPN degrees using the linguistic terms “normal state”, “borderline state”, “clear-cut pathology” and “severe pathology”. The fuzzy model showed a 90% concordance with the medical diagnosis, but electroneuromyography was used as an identification parameter of the disease status. In addition to the fact that this neurological assessment is of difficulty access, the disease detection will only occur when the degeneration of the nerve fibers is already present and the alterations are irreversible.

More recently, Picon *et al.* (2012) developed a classification model based on the evaluation of the most common signs and symptoms of DPN, HbA1c levels and diabetes disease duration. The model gives scores between 0-10 for the DPN degree and classifies the patients between mild, moderate and severe neuropathic individuals. Watari *et al.* (2014) refined this model using as inputs (i) the presence of typical DPN symptoms, based on Michigan Neuropathy Screening Instrument (Feldman *et al.*, 1994), (ii) tactile sensitivity, evaluated with 10-g Semmes-Weinstein monofilament

under 4 areas of both feet (Perkins *et al.*, 2001; Bakker, Apelqvist and Schaper, 2012) and (iii) vibratory perception, evaluated with a 128-Hz tuning fork (Perkins *et al.*, 2001). The model classified the patients in absent, mild, moderate and severe DPN, and presented a very strong correlation with the experts' opinion (Pearson's coefficient $r = 0.94$) and a high accuracy level when classifying patients as having DPN (ROC curve area = 0.91).

Therefore, the identification of different degrees of DPN based on a fuzzy logic, that takes into account the uncertainties of classification, has fundamental importance in the investigation of the biomechanics consequences of DPN progression to avoid misinterpretation of the results based on the heterogeneity in the experimental groups.

1.3. Muscle activation pattern of diabetic neuropathic individuals and novel tools for electromyography assessment

The motor dysfunction caused by DPN interferes with muscular activity of the lower limbs while performing daily living tasks (Abboud, Rowley and Newton, 2000; Kwon *et al.*, 2003; Sacco and Amadio, 2003; Akashi *et al.*, 2008; Sacco, Akashi and Hennig, 2010; Gomes *et al.*, 2011; Onodera *et al.*, 2011; Watari *et al.*, 2014). The existing literature has shown altered activity at the ankle muscles in the presence of DPN during gait stance phase, reporting a delayed (Akashi *et al.*, 2008; Sacco, Akashi and Hennig, 2010) and lower activation (Kwon *et al.*, 2003) of gastrocnemious muscles, and a delayed activation of tibialis anterior muscle (Abboud, Rowley and Newton,

2000; Sacco and Amadio, 2003). Altered activity at the knee muscles, especially the extensors, have also been found on DPN individuals. Studies have described a delayed peak activity and a lower activity of the vastus lateralis muscle at heel strike during gait (Abboud, Rowley and Newton, 2000; Kwon *et al.*, 2003; Sacco and Amadio, 2003; Akashi *et al.*, 2008; Sacco, Akashi and Hennig, 2010; Watari *et al.*, 2014) and an earlier onset at initial contact with a diminished activity was reported during pre-swing phase for rectus femoris (Sawacha, Spolaor, *et al.*, 2012). Altered activity of vastus lateralis has also been described during stair descent/ascent (Onodera *et al.*, 2011), suggesting that the presence of DPN alters the muscle coordination in different locomotor tasks.

However, the surface electromyography (sEMG) results are conflicting, inconsistent (Fernando *et al.*, 2013), based on single or bipolar recordings, and are still insufficient to understand the motor control alterations that occur as a consequence of DPN. Contributions have been made in the description of gait variables in DPN-population, as alterations in the spatio-temporal variables (Courtemanche *et al.*, 1996; Katoulis *et al.*, 1997; Kwon *et al.*, 2003; Menz *et al.*, 2004), kinematics, ground reaction forces (Katoulis *et al.*, 1997; Shaw *et al.*, 1998; Sacco and Amadio, 2000; Sacco *et al.*, 2015) and plantar pressures (Lavery *et al.*, 2003; Bacarin, Sacco and Hennig, 2009; Sacco *et al.*, 2009, 2014; Ko, Hughes and Lewis, 2012; Sawacha, Guarneri, *et al.*, 2012), but one should consider that (i) the comprehension of the internal physiological phenomena (muscular action) that is responsible for the locomotor external patterns observed in this population is still incomplete and (ii) the literature is still inconclusive in respect to the effects of the disease progression or the different degrees of DPN on the biomechanical variables.

The high-density sEMG (HD-sEMG) allows the use of more than one detection point along the muscle fibers, since it uses more than two electrodes to acquire the muscular activity signal. The system detection can be made of a linear array of electrodes or of a 2-D linear array, composing a matrix (Merletti *et al.*, 2010). The detection points can be allocated perpendicular, transversal or in both directions in relation to the muscle fibers (Gazzoni, Farina and Merletti, 2004). Signals can be collected in a monopolar way, meaning that each signal from each electrode is referenced to the same reference electrode, or can be collected based in a spatial differential filter. The monopolar signals can also be spatially filtered off-line, generating new signals that are a result of a linear combination of the detected signs respecting a specific geometrical configuration (Reucher, Rau and Silny, 1987; Disselhorst-Klug, Silny and Rau, 1997; Karlsson *et al.*, 2009). Therefore, the use of HD-sEMG has the potential to amplify the study of muscular activation patterns.

The analysis of single channel signals is usually based on the statistical analysis of discrete variables obtained from linear envelopes of the sEMG signal, or on the qualitative analysis of envelop patterns, and that is what has been made in DPN population for more than 15 years. The use of multiple detection channels provides a better reliability of sEMG signal (Merletti, Farina and Gazzoni, 2003; Farina and Merletti, 2004) and allows the acquisition of signals of larger muscle areas, being more representative of its whole activity. It also allows the extraction of information about the MU's conduction properties, innervation zones locations, length of muscle fibers, motor unit frequency rates and fiber recruitment strategies (Merletti, Holobar and Farina, 2008; Holobar *et al.*, 2009; Karlsson *et al.*, 2009; Mesin, Merletti and Rainoldi,

2009). Diseases that cause neurophysiological alterations as DPN would benefit from this approach since it provides additional information on the control mechanisms of muscular contractions and force generation (Merletti, Farina and Gazzoni, 2003; Drost *et al.*, 2006).

More recently, two studies assessed the vastus lateralis muscle activity with HD-sEMG in diabetic individuals. These studies found a heterogeneity in spatial sEMG distribution (Watanabe *et al.*, 2012) and lower mean MU instant firing rate during ramp contractions (Watanabe *et al.*, 2013), showing that diabetes alters the patterns of muscle recruitment. However, the effects of DPN or its severity were not assessed. Also, studies evaluating muscle fiber conduction velocity (MFCV) in subjects with DPN during isometric maximal contractions described lower MFCV values for vastus lateralis (Sacchetti *et al.*, 2013; Butugan *et al.*, 2014) in DPN patients, but no differences were found in biceps femoris MFCV between subjects with and without DPN (Butugan *et al.*, 2014).

The study of maximal contractions is reasonable, but during this kind of contraction a lot of MU are recruited and subtle differences in the motor control that can occur due to the loss of MU, an established fact in DPN individuals, might not be so easily detected. In addition, most of the daily living tasks require activation levels below 30% of maximal voluntary contractions.

In an interesting approach, Suda *et al.* (2016) investigated the effects of different degrees of DPN in MFCV during gait, reporting lower vastus lateralis MFVC in diabetics with or without DPN, regardless of DPN degree, during the early stance and propulsion phases of gait. The same study described a progressive increase in MFCV of

biceps femoris with DPN severity both in early stance and swing phases of gait. Although this study showed interesting results, we should be careful when interpreting them, because the calculation of MFCV during dynamic contractions has some reservation, as, for example, the the shift of the electrodes relative to muscle fibers (Farina, 2006).

The study of isometric contractions have been explored as a simplified model of the physiological mechanism that underlies the control of movement (Enoka *et al.*, 2003). Particularly, the study of sEMG signals during low-level isometric contractions allows a better understanding of how motor control can be affected in DPN since it is a task that recruits mainly type I fibers (Duchateau and Enoka, 2011), which are more affected by DPN. Also, the use of HD-sEMG allows the assessment of muscle activation patterns over a large area (Samani *et al.*, 2010).

1.4. Dynamical systems approach in the analysis of biological signs: motor complexity

The dynamics of different physiological processes are complex. Biological systems are regulated by mechanisms that operate over multiple scales in time and space (Costa, Goldberger and Peng, 2005). The complexity of a biological system reflects its ability to adapt and function in an environment that is constantly changing (Peng, Costa and Goldberger, 2009; Manor *et al.*, 2010). This complexity arises from the interaction of structural units and regulatory feedback loops that operate to enable the organism to adapt to this non-static environment (Goldberger, Peng and

Lipsitz, 2002; Peng, Costa and Goldberger, 2009). Thus, structural and/or functional alterations due to a disease status, as well as aging, would affect the interactions that are responsible for this constant adaption between the body and the mechanical demands of an environment, reducing the system's adaptive capacity and, consequently, degrading its biological complexity (Peng, Costa and Goldberger, 2009).

In biological data that represent physiological output, as force and EMG signals, complexity can be detected as multiple temporal scales present in the apparent irregular dynamics of the signal (Manor *et al.*, 2010). Motor complexity can be assessed by quantifying the amount of variability and the structure of this variability by nonlinear methods (Svendsen and Madeleine, 2010).

The amount of variability can be represented by the standard deviation (SD) of the outcome studied, since it provides an index of the degree of deviation from a point in a distribution of scores (Slifkin and Newell, 1999). It captures the magnitude of fluctuations in the system response. Variables as sample entropy (SaEn) and approximate entropy (ApEn), between others, measure variations in the system output along another dimension – time – that is independent of the signal magnitude. They measure, therefore, the way that the system output changes over time, which is the structure of the variability of a given biological signal or of the system dynamics (Slifkin and Newell, 1999; Richman and Moorman, 2000).

The use of variables based on entropy measures relies on concepts from Information Theory, in which the information content is quantified by the entropy. Thus, the higher the entropy information of a temporal series, the more complex it is (Duarte and Sternad, 2008). In this way, the algorithms based on entropy measure the

regularity of a temporal series (Costa, Goldberger and Peng, 2002). Pathological states and aging can be defined as a sustained exhaustion of large spectrum correlations, and loss of information. Thus, the disease dynamics is associated either with higher regularity resulting in variability decrease, or higher variability due to the loss of the correlation properties. Both situations would cause an entropy decrease (Costa, Goldberger and Peng, 2002).

Different entropy measures have been developed and applied in the analysis of biological signs. The most used ones for force and/or sEMG signals are ApEn and SaEn. The ApEn is a measure that was developed to quantify the degree of predictability of a data series. It is fundamentally a statistical measure of regularity of the signal, and not a direct index of physiological complexity (Goldberger, Peng and Lipsitz, 2002). The higher the ApEn of a signal, the more irregular it is, and as lower the ApEn, more regular or more periodical is the behavior. ApEn measures the probability that a certain configuration of a data subset inside a temporal series will allow to predict the configuration of another data subset inside the same temporal series, but that is allocated within a certain distance (Harbourne and Stergiou, 2009). However, ApEn presents some bias towards regularity, meaning that it suggests more similarity than it is actually present due to the way it calculates the entropy, repeating vectors of the time series (self-matched vectors) in the complete time series that is being analysed. This bias results in two main drawbacks – it heavily depends on the time series length and it does not show relative consistence (Richman and Moorman, 2000).

SaEn was developed to reduce the bias of ApEn calculation and also has the advantage of being a simpler measure (Richman and Moorman, 2000; Lake *et al.*,

2002). The main difference between ApEn and SaEn, is that SaEn does not use self-matched vectors in the calculus, as ApEn does. This reduces the bias shown by ApEn and makes SaEn a measure that better matches with the idea that entropy is truly reflecting the rate of new information being generated (Richman and Moorman, 2000). SaEn provides a measure of “orderly structure” within the time series since it tests if there are any repeated patterns of various lengths, including the ones that might not be repeating at regular intervals (Duarte and Sternad, 2008). The lower SaEn values are, the higher the similarity in the temporal series is and, therefore, less complex it is (Richman and Moorman, 2000).

The multiscale entropy (MSE) was developed based on the SaEn, allowing the use of SaEn in multiple temporal scales (Costa, Goldberger and Peng, 2002). It allows the computation of SaEn as a function of the temporal scale by adding it as a factor to the calculus. The original time series has a scale of one and new consecutive coarse-grained scales are constructed by different values of the scale factor (Figure 1), and SaEn is calculated for each coarse-grained series and plotted as a function of the scale factor (Costa, Goldberger and Peng, 2005). Figure 2 illustrates an example of MSE curves for biological data time series.

The calculus of MSE is an interesting approach if one considers that the mechanisms behind the biological complexity of a system also operate over multiple scales. The MSE calculation, however, is more time-consuming, making it harder to use on the analysis of multiple data as from HD-sEMG. It also needs longer time series to be computed, in contrast to SaEn, which is independent of data length.

Figure 1 – Schematic illustration of the coarse-grained procedure for scales 2 and 3. Each coarse-grained time-series is constructed by averaging the data points from non-overlapping windows. The number of data points utilized correspond to the scale factor. Based on (Costa *et al.*, 2003).

Figure 2 – MSE curves (mean $\pm$ standard error) for cardiac interbeat time series from 20 healthy young subjects and 20 healthy elderly subjects. Based on (Costa, Goldberger and Peng, 2002).

The study of the complexity of a system can add information about the complicated and rich mechanisms of information processing and integration necessary for an optimal motor control. Exploring the complexity of force generation and muscular activation in DPN individuals, where a series of structural and functional

neuromuscular alterations occur, would bring a deeper understanding of the different neuromuscular responses that can be adopted due to the disease status. Also, examining the complexity of the neuromuscular system of DPN-subjects through its progression, by examining subjects with different degrees of DPN, may bring new insights about the temporal effect of the disease on motor control.

2. OBJECTIVES

The general purpose of this study is to contribute for a better understanding on how motor control of the lower limbs is affected in DPN individuals. Fluctuations of the force and sEMG signal during voluntary contractions have been studied as a simplified model of the physiological data mechanism that underlies the control of movement. The complexity analysis of the muscular recruitment patterns from lower limbs, obtained by HD-sEMG, together with the corresponding force outputs from knee and ankle joints, would add to the knowledge of the disease effects in the motor control. Additionally, examining the complexity of force and muscle activity of DPN individuals along the progression of the DPN, from subclinical to the more severe stages, will help understanding how the possible alterations will develop with DPN progression, which in turn will help the DPN management, adding information that can be useful in building new rehabilitation strategies. The ankle and knee joints have important roles during locomotion tasks, and alterations in their control will certainly affect daily life activities, therefore, restoring neuromuscular control of these joints in this population has an important clinical relevance.

2.1 Main aim

The main aim of this study is to investigate the complexity of the neuromuscular system of DPN subjects by evaluating the amount and structure of

variability of sEMG patterns and force data during low-level isometric contractions in diabetic patients with different degrees of DPN, classified by a fuzzy-logic based system.

2.2 Specific aims

The specific aims are:

- Evaluate and compare the complexity (amount and structure of variability) of force during dorsiflexion, plantarflexion, knee flexion and knee extension at low-level isometric forces (10%, 20% and 30% of maximal voluntary contraction) in diabetic individuals with different DPN degrees;
- Evaluate and compare the complexity (amount and structure of variability) of tibialis anterior, gastrocnemius medialis, vastus laterals and biceps femoris muscles activity at low-level isometric forces (10%, 20% and 30% of maximal voluntary contraction) in diabetic individuals with different DPN degrees.

2.3 Hypothesis

The hypothesis of this study is that DPN alters the motor complexity, expressed as an increased amount of variability and a decreased structure of variability; in sEMG and force outputs and that these alterations are aggravated by the severity of the DPN.

3. METHODS

The results of this study have already been published in two papers. Therefore, this thesis is being presented as a compilation of scientific papers, reproduced in the following sections.

The first study has been published in *Clinical Biomechanics* in 2017 (impact factor = 1.636) and presents the results obtained by the evaluation of the structure and amount of variability of force during dorsiflexion and plantarflexion, and sEMG signals from tibialis anterior and gastrocnemius medialis with multichannel sEMG (64-electrode matrix). Data was acquired during isometric contractions at 10%, 20% and 30% of MVCs (Suda *et al.*, 2017a).

The second study was also published in 2017 in *Muscle & Nerve* (impact factor = 2.713) and follows the same rationale as the previous one, with the difference that in this second paper the force data was obtained during knee flexion and knee extension, and sEMG signal were acquired from vastus lateralis and biceps femoris muscles (Suda *et al.*, 2017b).

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Reduced complexity of force and muscle activity during low level isometric contractions of the ankle in diabetic individuals

E.Y. Suda^a, P. Madeleine^b, R.P. Hirata^b, A. Samani^b, T.T. Kawamura^a, I.C.N. Sacco^{a,*}^a Laboratory of Biomechanics of Human Movement, Dept. Physical Therapy, Speech and Occupational Therapy, School of Medicine, University of São Paulo, São Paulo, Brazil^b SMI, Department of Health Science and Technology, Faculty of Medicine, Aalborg University, Denmark

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ABSTRACT

Background: This study evaluated the structure and amount of variability of surface electromyography (sEMG) patterns and ankle force data during low-level isometric contractions in diabetic subjects with different degrees of neuropathy.

Methods: We assessed 10 control subjects and 38 diabetic patients, classified as absent, mild, moderate, or severe neuropathy, by a fuzzy system based on clinical variables. Multichannel sEMG (64-electrode matrix) of tibialis anterior and gastrocnemius medialis muscles were acquired during isometric contractions at 10%, 20%, and 30% of the maximum voluntary contraction, and force levels during dorsi- and plantarflexion were recorded. Standard deviation and sample entropy of force signals were calculated and root mean square and sample entropy were calculated from sEMG signals. Differences among groups of force and sEMG variables were verified using a multivariate analysis of variance.

Findings: Overall, during dorsiflexion contractions, moderate and severe subjects had higher force standard deviation and moderate subjects had lower force sample entropy. During plantarflexion, moderate subjects had higher force standard deviation and all diabetic subjects had lower entropy. Tibialis anterior presented higher root mean square in absent group and lower entropy in mild subjects. For gastrocnemius medialis, entropy was higher in severe and lower in moderate subjects.

Interpretation: Diabetic neuropathy affects the complexity of the neuromuscular system during low-level isometric contractions, reducing the system's capacity to adapt to challenging mechanical demands. The observed patterns of neuromuscular complexity were not associated with disease severity, with the majority of alterations recorded in moderate subject.

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1. Introduction

In 2015, the International Diabetes Federation reported that approximately 415-million individuals around the world suffered from diabetes, with approximately 642-million individuals predicted to be affected by the disease by 2040 (IDF, 2015).

Diabetic peripheral neuropathy (DPN) is associated with a high risk of foot ulcers and lower limb amputation (Malyar et al., 2016; Veves et al., 1992). DPN affects approximately 50% of all the diabetic population (Tesfaye et al., 2010). It affects both sensitive and motor fibers of the limbs, with distal to proximal progression (Callaghan et al., 2012; England et al., 2005). A better understanding of DPN-induced motor dysfunction is needed for the management of this co-morbidity.

Although the first symptoms of DPN are sensorial, some studies have provided evidence for alterations in motor neurons in the early stages of

the disease (Meijer et al., 2008). In addition to motor neuron impairment, diabetes interferes with the properties of skeletal muscles (Ijzerman et al., 2011), leading to marked decreases in the muscle mass of the lower limbs (Andersen et al., 2004a; Andreassen et al., 2009), lower levels of muscular activation (Onodera et al., 2011; Watari et al., 2014) and poorer ability to generate force (Allen et al., 2014b; Andersen et al., 2004b; Orlando et al., 2015). Concomitant with these changes, DPN-induced structural and functional neuromuscular alterations (Orlando et al., 2015) may influence motor control and one of its constituent - motor variability, i.e. system complexity (Srinivasan and Mathiassen, 2012).

Motor complexity can be assessed by quantifying the amount of variability (i.e., standard deviation) and the structure of this variability by nonlinear methods (Svendsen and Madeleine, 2010). The magnitude of variability, represented by the standard deviation (SD) of the outcome studied, refers to the deviation from the desired system response or the magnitude of inherent fluctuations in the system response (Sliifkin and Newell, 1999). Sample entropy (SaEn) is a nonlinear measure of the structure of variability or complexity of a given biological

* Corresponding author at: Departamento de Fisioterapia, Fonoaudiologia e Terapia Ocupacional, Cidade Universitária, R. Cipotânea, 51, São Paulo 05360-160, Brazil.
E-mail address: icnsacco@usp.br (I.C.N. Sacco).

signal (Richman and Moorman, 2000). SaEn has been used to quantify the complexity of force (Chow and Stokic, 2014; Pethick et al., 2015; Svendsen et al., 2011; Svendsen and Madeleine, 2010) and sEMG time series (Enders et al., 2015; Mista et al., 2015; Rathleff et al., 2013, 2011) to obtain a better understanding of the time-dependent structure of biomechanical signal variability. Thus, exploring biomechanical data, such as sEMG and force signals, can improve our understanding of motor-control strategies adopted in the presence of different degrees of DPN. In the literature, a disease status has been associated with a loss of complexity due to the loss of system elements and/or their functional interactions (Hong and Newell, 2008; Vaillancourt and Newell, 2002; van Emmerik and van Wegen, 2002), and separate control processes can influence the amount and structure of force variability (Sosnoff et al., 2006).

This study investigated the complexity of the neuromuscular system of DPN subjects by evaluating the amount and structure of variability of sEMG patterns and force data obtained during low-level isometric contractions. We hypothesized that DPN would result in altered motor complexity, revealed by decreased structure of variability of sEMG and force data and that these alterations would be aggravated by the severity of the disease. Additionally, we hypothesized that the disease status would result in an increased amount of variability of force data and sEMG patterns (Lipsitz, 2002).

2. Methods

2.1. Subjects

Thirty-eight adult subjects with diabetes mellitus were divided into four groups: 12 without DPN, (absent group), 11 with mild DPN (mild group), 7 with moderate DPN (moderate group) and 8 with severe DPN (severe group). The disease severity was classified as described below. All the subjects had type 2 diabetes, except one within the severe DPN group who had type 1 disease. Ten healthy nondiabetic individuals served as the control group. The participants' anthropometric and clinical data are shown in Table 1.

All participants should not have any of the following: vestibulopathy; severe visual deficits; partial or total limb amputations; Charcot arthropathy; plantar ulceration at the time of the evaluation; neurological or orthopedic impairments due to stroke, Parkinson's disease, or cerebral palsy; poliomyelitis; rheumatoid arthritis, or other neuropathies; severe nephropathy causing edema; or signs of ischemia (an ankle-brachial index above 0.6) (Tesfaye, 2006).

All patients underwent a clinical evaluation by an experienced physiotherapist that assessed DPN symptoms, tactile sensitivity, vibratory perception to classify the severity status. All these clinical parameter were used as linguistic inputs in a fuzzy model developed by Watari et al. (2014). The DPN degree score (x) was sorted with the following division: (i) $x \leq 2.0$ means absence of DPN; (ii) $2.0 < x \leq 4.5$ means mild DPN; (iii) $4.5 \leq x \leq 7.5$ means moderate DPN, and (iv) $x > 7.5$ means severe DPN. Patients with a history of ulceration were automatically classified as severe DPN (Veves et al., 1992).

All participants provided their signed informed consent prior to inclusion. The study was approved by the local ethics committee (protocol number 187/13) and conformed to the principles of the Declaration of Helsinki.

2.2. Experimental procedures

2.2.1. Experimental protocol

The sEMG recordings were performed on the dominant leg. After placement of the sEMG matrix (described below), the subjects were seated in an upright position, with the evaluated leg positioned in a customized ankle dynamometer. Each subject's knee was fully extended, and the ankle was in a neutral position at 90° (Fig. 1).

The experiment was performed by a physical therapist blinded to the subject's classification, always in the same order: (i) sEMG and dorsiflexion force recordings of the tibialis anterior (TA) muscle and (ii) sEMG and plantarflexion force measurements of the gastrocnemius medialis (GM) muscle. The experiment proceeded as follows:

1. The participants performed a familiarization task (dorsiflexion or plantarflexion) of the required movement, aiming to achieve the requested force level using a visual feedback system.
2. Maximal voluntary contraction (MVC) was recorded twice (3-s, 1-min pause) to determine the subject's maximal ankle force (dorsiflexion or plantarflexion). The maximal peak force level achieved during both trials was used as the reference in subsequent recordings.
3. Low-level force recordings were obtained of six isometric contractions (10-s each, 1-min pause) always performed in the following order: two contractions at 10% MVC, 20% MVC, and 30% MVC. These three different force levels were chosen to study how the recruitment of additional motor units would influence motor variability with the presence of DPN. Visual feedback was provided, and an error of $\pm 2\%$ from the target level was accepted. The recordings started after the participant was able to stabilize and maintain the required force level for the task.

Two subjects (one with moderate DPN and one with severe DPN) were not able to perform the tasks. Thus, in the first case, only the TA data recorded during dorsiflexion were used. In the second case, only the GM data recorded during plantarflexion were considered.

2.2.2. High-density sEMG and force recordings

Force was measured with a strain-gauge load cell (traction/compression, 100-kg range) in a customized dynamometer (NEG1, OT Bioelettronica, Turin, Italy). The rotational axis of the ankle joint was aligned with the center of rotation of the dynamometer. The subject's foot was firmly strapped to the footplate, which was connected to the strain gauge transducer using straps.

High-density sEMG recordings were acquired from the TA and GM muscles 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,

Table 1
Anthropometric variables and clinical data for the experimental groups, reported as mean (standard deviation) or number (%).

	Control (n = 10)	Absent (n = 12)	Mild (n = 11)	Moderate (n = 7)	Severe (n = 8)	P
Age (years)	49.4 (9.6) ^c	58.9 (5.5)	59.2 (4.4)	60.9 (5.6)	60.1 (3.0)	0.001 ^a
Sex (% male)	40	25	54.5	57.1	62.5	0.430 ^b
Body mass index (kg/m ²)	26.0 (3.4)	26.3 (4.1)	29.3 (6.2)	29.1 $\pm$ 3.1	29.4 (5.7)	0.284 ^b
Diabetes mellitus duration (years)	–	11.9 (6.7)	9.9 (7.6)	15.3 (4.7)	18.6 (17.6)	0.279 ^a
Fasting glucose level (mg/dL)	–	128.3 (42.6)	132 (29.3)	165 (70.5)	179.5 (117.3)	0.426 ^a
Neuropathy degree score	0.63	1.0 (0.6)	3.1 (0.9) ^c	5.1 (0.4) ^c	8.1 (1.2) ^c	<0.001 ^a

^a ANOVA test.

^b Chi-square test.

^c Group statistically different from all others – Bonferroni pairwise comparisons.

Fig. 1. Experimental setup illustrating the electrode matrix placed in tibialis anterior for sEMG recordings with the ankle positioned at 90° in the dynamometer (A). The figure also illustrates the placement of the electrodes matrix in the tibialis anterior (B) and gastrocnemius medialis muscle (C).

the skin was shaved, lightly abraded, and cleaned. To determine the matrix location, active contractions against the examiner manual resistance were performed. The matrix was positioned with the columns along the longitudinal axis of the muscles, according to anatomical landmarks (Houtman et al., 2003). A conductive cream was inserted into the cavity of the foam to assure proper electrode-skin contact. The reference electrode was fixed at the calcaneus tendon.

The sEMG signals were amplified 1000 times, sampled at 2048 Hz (256-channel sEMG amplifier, EMG-USB2+, OT Bioelettronica, Torino, Italy; -3 dB bandwidth 10–500 Hz), and digitized with a 12-bit A/D converter. The force signals were amplified 100 times, fed to the auxiliary inputs of the EMG-USB2+, and sampled synchronously with the sEMG signals.

2.3. Force data analysis

Force data analysis was performed off line with MATLAB R2016a software (MathWorks, MA, U.S.). The force data were low-pass filtered at 10 Hz (4th order). For the MVC trials, the mean force was computed over 500-ms windows with a 100-ms overlap, and the highest value was considered the maximum force. The window time at which the maximum force occurred was also computed for further analysis.

For low-level force recordings (10, 20, and 30% MVC), the amount of force variability was quantified by the SD of the exerted force over the 5 s central epoch (2.5–7.5 s) of activity during a 10-s trial. The force signal was visually inspected. If this signal was not in the $\pm 2\%$ range, a new 5-s window with a more stable force signal was selected for inclusion in the analysis. The selected window was used for all further force data and sEMG analyses.

A nonlinear analysis was also performed to assess the structure of force variability. The SaEn of the exerted force was calculated over the 5 s central epoch (2.5–7.5 s) of activity during the 10 s trial, and for this purpose the time series were down sampled by ten and the embedding dimension (m) and the tolerance distance (r) were set to $m = 2$ and $r = 0.2 \times \text{SD}$ of the force channel (Svendsen and Madeleine, 2010). An increased SaEn indicates that the complexity of signal has increased or its predictability is reduced (Richman and Moorman, 2000).

2.4. High-density sEMG data analysis

The recorded monopolar sEMG signals were off-line band-pass filtered (10–500 Hz, 4th order Butterworth filter). A notch filter of 60 Hz and its 7 subsequent harmonics were applied to diminish the interference of the power line. The noisy channels were visually inspected and reconstructed based on the interpolation of the signals from the two neighbor-pairs channels. Fifty-nine bipolar sEMG signals were obtained along the columns of the electrodes (12×5 bipolar recordings, with one missing electrode), considering the longitudinal axis of the muscles. At each force level, the root mean square (RMS) values of the bipolar sEMG signals were computed over the same epoch used to compute the force level, providing RMS maps for each task. RMS values of the MVCs were also obtained for the epoch corresponding to the maximum contraction. The mean value of the RMS map of the MVCs was used as a reference value. The mean RMS obtained from each map was normalized to that of the reference value obtained from the MVCs. The normalized values were considered as the level of activation of the studied muscle.

SaEn values were computed for sEMG data (time series down sampled by two, $m = 2$ and $r = 0.2 \times \text{SD}$) to characterize the complexity of the muscle activity. The SaEn was calculated for 1 s extended epochs without overlap and for each bipolar derivation during the 5-s epoch from the 10-s trial. The mean of the SaEn obtained from the five epochs was calculated, and a SaEn map was produced. The mean SaEn in the map was considered the level of complexity.

2.5. Statistical analysis

Multivariate analysis of variance (MANOVA) of the dorsiflexion force variables (referred to hereafter as SD force and SaEn force) at all force levels (10%, 20%, and 30% MVC) was performed, with the independent factor being the level of DPN (control, absent, mild, moderate, and severe). When a significant multivariate effect was observed, a univariate ANOVA was performed using Bonferroni post-hoc tests corrected for pairwise comparisons. The same analysis was repeated for the plantarflexion variables (SD force and SaEn force), TA sEMG variables (RMS and sEMG SaEn), and GM sEMG variables (RMS and sEMG SaEn). In group comparisons, the effect sizes and their 95% confidence intervals (CIs) were estimated using Hedges' g (g), with g values between 0.2 and 0.5 considered small, those between 0.5 and 0.8 considered medium, and those above 0.8 considered large (Cohen, 1988). Analyses of covariance (ANCOVAs) were performed for all dependent variables (force SD, force SaEn, RMS and sEMG SaEn) using age as covariate in order to check the presence of age effects on neuromuscular control (Roos et al., 1997). The significance level was set as 5%. All statistical analyses were performed with IBM SPSS Statistics 23.0 (IBM, NY, U.S.).

3. Results

Age did not have a significant effect on the extracted parameters (ANCOVA P values between 0.06 and 0.967).

3.1. Mean forces

There were no statistical differences in the mean of maximum force values among groups for dorsiflexion (control = 537.3(248.7)N; absent = 544.8(199.4)N; mild = 504.7(208.3)N; moderate = 501.7(125.1)N; severe = 413.1(229.2)N; $F = 0.622$, $P = 0.650$) and plantarflexion (control = 633.3(257.3)N; absent = 651.4(244.0)N; mild = 551.5(132.8)N; moderate = 662.7(116.5)N; severe = 551.1(134.0)N; $F = 0.701$, $P = 0.596$).

3.2. Amount and structure of force variability during dorsiflexion

Fig. 2 shows the mean (SD) of the SD and SaEn force during dorsiflexion. The results of the ANOVA, effect sizes (g), and their corresponding CIs are reported in Supplementary material (Table S1). The MANOVA showed a significance between-group difference in dorsiflexion force values (Wilks' $\Lambda = 0.512$, $P < 0.001$). The ANOVA revealed a significant group effect on force SD at 10% MVC ($F = 5.41$, $P = 0.001$) and 20% MVC ($F = 4.820$, $P = 0.001$). Post-hoc comparisons showed that the SD force was significantly higher in those with moderate and severe DPN at 10% MVC with high practical significance (g). At 20% MVC, moderate DPN showed also higher SD force than controls and mild DPN ($P < 0.05$) with g values showing a higher SD in moderates compared to controls, absents and milds (large effect sizes).

The ANOVAs also showed a significant group effect on force SaEn at 10% ($F = 4.339$, $P = 0.003$), 20% ($F = 2.852$, $P = 0.028$) and 30% MVC ($F = 2.680$, $P = 0.037$). Post-hoc comparisons showed that at 10% and 20% MVC, moderate DPN had lower SaEn values than controls ($P < 0.05$). At 30% MVC, the SaEn of moderate group was significantly lower than that of absent group ($P < 0.05$). The effect sizes showed that the moderate DPN had lower SaEn than the other groups (medium and large effect sizes for all pairwise comparisons) at all the force levels studies.

3.3. Amount and structure of force variability during plantarflexion

Fig. 3 shows the mean (SD) of the SD and SaEn force during plantarflexion. ANOVA's results, the effect sizes (g) and their corresponding CIs with respect to the SD and SaEn at 10, 20, and 30% MVCs are reported in Supplementary material (Table S2). The MANOVA showed significant between-group differences in plantarflexion force values (Wilks' $\Lambda = 0.651$, $P = 0.039$). For SD force, ANOVA revealed a significant group effect at 30% MVC ($F = 2.742$, $P = 0.033$), and post-hoc comparisons showed that the Force SD of the moderate DPN group was higher than that of the controls ($P < 0.05$). For SaEn force, the ANOVA revealed a significant group effect at 30% MVC ($F = 5.040$, $P = 0.001$), and post-hoc comparisons showed that the SaEn of the diabetic groups were lower than controls at 30% MVC ($P < 0.05$). At 30%

Fig. 2. Mean (SD) of force standard deviation (SD, N) and force sample entropy (SaEn, arbitrary unit) during dorsiflexion at 10% MVC, 20% MVC and 30% 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. *Statistically differences detected in post-hoc tests.

Fig. 3. Mean (SD) of force standard deviation (SD, N) and force sample entropy (SaEn, unitless) during plantarflexion at 10%, 20% and 30% 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. *Statistically differences detected in post-hoc tests.

MVC, effect sizes demonstrated that the SD values of all the diabetic groups were higher than controls, whereas the SaEn values of the diabetic groups were lower (medium and large effect sizes in all pairwise comparisons).

3.4. Level of muscular activation and structure of sEMG variability during dorsiflexion

Fig. 4 shows the mean (SD) of the RMS and SaEn of the TA at 10, 20, and 30% MVC. The results of the ANOVAs and effect sizes (g), with their corresponding CIs, are reported in Supplementary material (Table S3). The MANOVA showed significant between-group differences in sEMG variables (Wilks' $\Lambda = 0.576$, $P = 0.002$). The ANOVAs revealed a significant group effect for RMS values at 10% MVC ($F = 2.729$, $P = 0.034$) and 20% MVC ($F = 2.754$, $P = 0.033$). At both 10% and 20% MVCs, as shown by the post-hoc comparisons, RMS values of the absent group were higher than controls ($P < 0.05$). The g values showed that all diabetic groups had higher RMS than controls (medium and large effect sizes in all the pairwise comparisons). With respect to SaEn values, ANOVAs showed a significant group effect at 10% MVC ($F = 4.912$, $P = 0.001$), 20% MVC ($F = 2.877$, $P = 0.027$) and 30% MVC ($F = 3.158$, $P = 0.018$). Post-hoc comparisons at 10% MVC demonstrated that all diabetics had significantly lower SaEn than controls ($P < 0.05$). At both 20

and 30% MVCs, SaEn of the mild DPN group were significantly lower compared to controls. The effect sizes showed that the SaEn TA values of all diabetic groups were lower compared to control group (medium and large effect sizes in all the pairwise comparisons) at all force levels.

3.5. Level of muscular activation and structure of sEMG variability during plantarflexion

Fig. 5 shows the mean (SD) of RMS and SaEn for the GM at 10, 20, and 30% MVCs. ANOVAs results, Hedges' effect sizes, with their corresponding CIs, are reported in Supplementary material (Table S4). MANOVA revealed significant between-group differences among the sEMG variables (Wilks' $\Lambda = 0.554$, $P = 0.001$). As shown by the ANOVAs, the group effect was significant only for SaEn values at 10% MVC ($F = 4.031$, $P = 0.005$) and 20% MVC ($F = 3.055$, $P = 0.021$). The post-hoc comparisons showed that SaEn of severe group at 10% MVC was higher than control and moderate groups ($P < 0.05$). At 20% MVC, post-hoc comparisons showed that the SaEn of the severe group was higher than moderate group ($P < 0.05$). As demonstrated by the g values, at 10% MVC, the severe group had higher SaEn, and the moderate group had lower SaEn at 20% MVC (medium and large effect sizes in all the pairwise comparisons).

Fig. 4. Mean (SD) of RMS (% MVC) and sEMG sample entropy (SaEn, unit less) for tibialis anterior muscle during dorsiflexion at 10% MVC, 20% MVC and 30% MVC. Lines and circles represent desired 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.

4. Discussion

We found an increased amount of force variability and lower structure of force variability during both tasks in subjects with DPN. The DPN subjects showed lower EMG complexity, although the SaEn value of the GM was increased in those with severe DPN at 10% MVC during plantarflexion. The observed changes did not follow a pattern of progressive alteration in accordance with the disease severity, as the alterations were more evident in moderate DPN.

To maintain a constant low level of force, the neuromuscular system has to be capable of perceiving very small force oscillations and correcting these oscillations. We expected/hypothesized that with the progression of DPN, higher variability in the force output would occur. In line with our expectation/hypothesis, this was actually observed in the present study. During dorsiflexion, (i) the force output of the moderate and severe DPN groups (i.e., those with later-stage disease) showed a higher amount of variability than controls, DPN absent group, and mild DPN group at 10% MVC. (ii) With the increase in the force demand (20% MVC), the moderate DPN group still exhibited higher force variability than the less affected subjects, whereas the force variability of the severe DPN group was only higher than the control group. (iii) With another increase in the dorsiflexion force (30% MVC), these differences were no longer present, although the moderate DPN group showed a tendency toward higher force variability than the

control and mild DPN groups. During plantarflexion, the amount of force variability was higher at 30% MVC in all diabetic groups compared with controls. Previous studies reported less force output variability in muscles with large number of motor units (Hamilton et al., 2004; Sosnoff and Newell, 2006). Evidences that DPN causes a loss of motor units have previously been described (Allen et al., 2013, 2014a; Souayah et al., 2009). Such loss could contribute to the augmented force variability. Besides that, the distal muscles (e.g., TA and GM) of DPN individuals would be expected to show higher force output variability while producing torque due to the loss of muscle mass and strength (Andersen, 2009; Andersen et al., 2004b; Moore et al., 2015). This was corroborated during dorsiflexion at 10 and 20% MVCs and plantarflexion at 30% MVC.

The alterations observed in the present study in the structure of force variability followed almost the same pattern observed in the alterations in the amount of force variability, but with an inverted order. During dorsiflexion, at 10 and 20% MVCs, structural force variability was reduced in the moderate DPN group. During plantarflexion, structural force variability was reduced at 30% MVC in all diabetic groups (absent, mild, moderate, and severe). Slifkin and Newell (1999) showed that the structure of force variability reflected the information transmitted during the performance of a task, meaning that improvements in the performance occur with an increase in the structural variability of the system output. In addition, studies demonstrated that as the structural

Fig. 5. Mean (SD) of RMS (%MVC) and sEMG sample entropy (SaEn, unit less) for gastrocnemius medialis muscle during plantarflexion at 10%MVC, 20%MVC and 30%MVC. Lines and circles represent desired 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. * Statistically differences detected in post-hoc tests.

variability increased, the system adaptability also increased and that this capacity was important in maintaining the ability to respond to internal and external perturbations (Lipsitz, 2002). In the present study, the lower structure of force variability in DPN underlined the effect of the disease on motor control during isometric contractions. This lower structure might also have been due to the loss of motor units. If fewer motor units are available, the response will be more stereotyped, and force output, more regular along time. Both plantar and dorsiflexion movements are very important for locomotor activities, such as walking. Thus, it is conceivable that the observed alterations in the variability of the force signals would compromise daily living activities in the presence of DPN. Further studies investigating variability in force signals in those with DPN during activities of daily living are warranted.

DPN reduced the structure of sEMG variability of TA activity. At 10% MVC, TA showed lower structure of variability of muscle activity pattern in all diabetic groups compared to control group, regardless of the presence or severity of DPN, compared to healthy subjects. With an increase in the force level (20 and 30% MVCs), this difference was more evident between healthy subjects and those with mild DPN. Allen et al. (2015) evaluated the intramuscular activity of TA in DPN subjects during isometric contractions at 25% MVC. These authors have also showed that DPN individuals had higher activity and fewer motor units activated when compared to controls. According to concepts of physiological complexity (Goldberger et al., 2002; Lipsitz, 2002), if reduced degrees of freedom of the motor system are available (fewer MU), a less

complex signal output would be generated, as observed in the present study. Additionally, the absent DPN group showed higher TA muscle activity levels when compared to healthy subjects at 10% and 20% MVC. This result showed that diabetes, despite the presence of DPN, played a key role in the performance of the TA. Higher sEMG signals amplitude is related to the recruitment of additional motor units and possibly changes in the firing properties of active motor units (Duchateau and Enoka, 2011). The clinical progression of diabetes causes alterations in the structure and composition of muscle proteins (Bozkurt et al., 2010), compromising the muscle quality, defined as the strength per unit mass, and the contractile quality, already described in the TA muscle of diabetic patients (Ijzerman et al., 2011; Moore et al., 2015). Thus, an increased sEMG amplitude could represent a compensatory mechanism that occurs in response to structural changes in the muscles of diabetic subjects.

As unexpected, at 10 and 20% MVCs, the GM sEMG data of severe patients showed more complexity when compared to control and moderate groups. In controls, the complexity of muscle activation decreased as the force level increased. The opposite behavior was observed in the severe patients, with higher muscle activity complexity at 10% MVC. The higher SaEn values can indicate adjustments to compensate for the motor and sensorial deficits due to the DPN with an increase in the complexity of muscle activity. As the force level increased, the recruitment pattern would also need to change to perform these adjustments. However, this mostly would not be possible in the case of the most severe subjects due to the losses caused by progressive denervation.

A novel and important finding was that motor variability seemed to be more affected in moderate subjects, while severe cases showed an apparent recovery of motor-control strategies. As DPN is progressive, the observed pattern of alteration suggests that in the moderate stage where the DPN is really installed, the effects of the disease on motor variability are most apparent. Similar findings have been reported in relation to pain stages with increases in motor variability being found in the acute stage of pain, while during subchronic and/or chronic stages the motor variability decreases (Madeleine, 2010; Madeleine et al., 2008). One of the responses to denervation caused by DPN is axonal sprouting, which allows muscle fiber reinnervation of intact neighboring motor units (Ramji et al., 2007). Although studies have provided evidence that the compensatory effects of axonal sprouting are limited (Allen et al., 2013; Ramji et al., 2007), such effects might have contributed to the recovery in the amount and structure of force variability in the severe group closer to the ones observed in the healthy participants in the present study.

In the present study, the amount and structure of force variability during isometric contractions changed according to the level of force exerted. Further increases in the force level induced increments in the amount of force variability, while the structure changes according to an inverted U-shaped function (Hong et al., 2007; Slifkin and Newell, 1999; Svendsen and Madeleine, 2010). This study evaluated low-level forces to explore the effects of DPN in motor control during the performance of a task that would mainly recruit type I fibers (Duchateau and Enoka, 2011). We evaluated two additional force levels to study the changes in motor control at low contraction levels. The current study clearly showed that low contraction levels (below 30% MVC) cannot be considered as an entity and that the level of muscle activation greatly influence the measured effects of DPN on motor activities. In addition, the small sample size of the moderate and severe groups could have masked the existence of other alterations due to DPN, although it was possible to detect some significant differences between the studied groups. Furthermore, the system used to classify the severity of DPN took into account signs and symptoms related to the sensorial aspects of DPN. Nevertheless, although the fuzzy system has been shown to be highly accurate when classifying the severity of DPN patients (Watarai et al., 2014), motor alterations may not follow the same path as sensorial ones. The classification system adopted may explain the failure to find a progressive effect of DPN according to the severity of the disease. Finally, future studies addressing the effects of DPN on sEMG activity should also compute index of co-contraction or mutual information among muscles pairs (Kellis et al., 2003; Madeleine et al., 2016).

5. Conclusion

Our findings showed that DPN affected the complexity of the neuromuscular system during isometric contractions of the ankle, reducing the capacity to comply with mechanical demands of low-level forces. We observed higher amount and lower structure of force variability, and lower structure of TA sEMG variability. The observed patterns were not associated with the DPN severity, with diabetic subjects with moderate DPN showing the majority of complexity alterations. We conclude that diabetes alone affects motor control, regardless of the presence of DPN.

Conflict of interest

No potential conflicts of interest relevant to this article were reported.

Authors' contributions

ICNS and EYS are the principal contributors to this work. As such, they had full access to all the data and take responsibility for the

integrity of the data and accuracy of the data analysis. ICNS, EYS, TTK, PM, RH, and AS were responsible for the study design, analysis and data interpretation, and manuscript literature review. TTK and EYS were responsible for the data collection. All the authors contributed to the manuscript and approved the final version. The material has not been and will not be submitted for publication elsewhere.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.clinbiomech.2017.01.001>.

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5. PUBLISHED PAPER 2

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

ENEIDA Y. SUDA, PT, MSc,¹ ISABEL C.N. SACCO, PhD,¹ ROGERIO P. HIRATA, PhD,² AFSHIN SAMANI, PhD,²

THIAGO T. KAWAMURA, PT,¹ and PASCAL MADELEINE, PhD, DSc

¹Laboratory of Biomechanics of Human Movement, Department of Physical Therapy, Speech and Occupational Therapy, School of Medicine, University of Sao Paulo, Sao Paulo, Brazil

²SML, Department of Health Science and Technology, Faculty of Medicine, Aalborg University, Fredrik Bajers Vej 7 D-3, 9220 Aalborg East, Denmark

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ABSTRACT: *Introduction:* This study evaluates 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 participants performed isometric contractions at 10%, 20%, and 30% of maximal voluntary contraction. Knee force and multichannel sEMG from vastus lateralis (VL) and biceps femoris were acquired. The SD of force and sample entropy (SaEn) of both force and sEMG were computed. *Results:* Participants with moderate DPN demonstrated high force-SD and low force-SaEn. Severely affected participants 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 participants in the later stages of the disease (moderate and severe) demonstrating most of the changes.

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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.¹ 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.² Loss of axonal function results in the loss of motor units and impaired axonal sprouting.³ 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 because of long-term loss of motor end plates.^{3,4} These alterations interfere with muscle strength and endurance while performing tasks of daily living.⁵ The literature has described altered activity in the knee muscles in the presence of DPN, especially in the knee extensors.^{6–14} However, most of these studies demonstrating changes in surface electromyography (sEMG) in relation to DPN are based on single-channel recordings.

High density sEMG with a grid of electrodes allows recording of the spatiotemporal muscle activity pattern.^{15,16} At low-level contraction forces, the assessment of the spatiotemporal pattern provides information on the variability in the motor unit recruitment pattern.¹⁷ Recording high-density sEMG also enables assessment of the level of complexity of the electromyographic activity over a large area.¹⁸ 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.^{19,20} Consequently, structural or functional alterations resulting from 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 for a deeper understanding of the different neuromuscular responses to disease status. Furthermore, examining the complexity of sEMG through the progression of the disease in persons 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 aging and disease.^{18,21} Therefore, assessing the complexity of motor control in participants with DPN during isometric

Additional supporting information may be found in the online version of this article.

Abbreviations: BF, biceps femoris; CI, confidence interval; DPN, diabetic peripheral neuropathy; MANOVA, multivariate analysis of variance; MVC, maximal voluntary contraction; RMS, root mean square value; SaEn, sample entropy; sEMG, surface electromyography; VL, vastus lateralis.

Key words: complexity; diabetes mellitus; force; high-density EMG; motor variability; sample entropy; surface electromyography

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Correspondence to: P. Madeleine; e-mail: pm@hst.aau.dk

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Diabetic Neuropathic Motor Control

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FIGURE 1. Experimental setup illustrating the electrode matrix placed at vastus lateralis for surface electromyography recordings with the knee positioned at 45° degrees (0° = full extension) in the dynamometer.

contractions performed at low-level contractions appears sound, especially because DPN affects mostly type I motor units, which are activated in these low-level tasks. This study evaluates 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 hypothesize that DPN will alter the motor complexity leading to an increased amount of variability and a decreased structure of variability in vastus lateralis (VL) and biceps femoris (BF) sEMG and in the knee extension and knee flexion force outputs, in line with previous studies.^{17,22}

MATERIALS AND METHODS

Participants. Thirty-eight individuals with diabetes mellitus were allocated to 4 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 participants had type 2 diabetes, except 1 from the severe group who had type 1 diabetes. Ten healthy, nondiabetic individuals were included as a control group. All participants gave signed informed consent. The study was approved by the Ethics Committee of the School of Medicine of the University of Sao Paulo (protocol No. 187/13) and was in conformity with the Declaration of Helsinki.

The inclusion criteria for diabetes participants were age between 40 and 68 years and diagnosis of diabetes type 1 or 2 with or without symptoms of DPN. The inclusion criteria

for control participants were 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 0.6,²³ Charcot arthropathy, history of poliomyelitis, rheumatoid arthritis or other neuropathies, and neurological or orthopedic impairments resulting from stroke, Parkinson disease, or cerebral palsy.

We classified the severity of the DPN (degree score 0–10) by a fuzzy system developed by Watari *et al.*¹¹ using 3 groups of clinical input variables: (1) tactile sensitivity, (2) vibratory perception, and (3) presence of typical DPN symptoms (as per Michigan neuropathy score²⁴). The DPN scores (x) were grouped as follows: (1) $x \leq 2.0$, absence of DPN; (2) $2.0 < x < 4.5$, mild DPN; (3) $4.5 \leq x \leq 7.5$, moderate DPN; and (4) $x > 7.5$, severe DPN. If ulceration history was present, the individual was automatically classified as severe DPN.²⁵

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, VL sEMG and knee extension force recordings followed by BF sEMG and knee flexion force measurement. These muscles were chosen to be representatives of knee flexion and extension that have been described as altered in diabetic patients.^{13,14} Prior to the sEMG and force assessments, the participants performed

Table 1. Anthropometric variables and clinical data (mean $\pm$ SD) for the experimental groups

Variables	Control (n = 10)	Absent (n = 12)	Mild (n = 11)	Moderate (n = 6)	Severe (n = 9)	P
Age (years)	49.4 $\pm$ 9.6*	58.9 $\pm$ 5.5	57.9 $\pm$ 5.9	60.3 $\pm$ 5.9	60.3 $\pm$ 2.8	0.002†
Sex (% male)	40	25	54.5	66.7	55.6	0.411‡
Body mass index (kg/m ²)	26.0 $\pm$ 3.4	26.3 $\pm$ 4.1	28.6 $\pm$ 5.4	29.5 $\pm$ 3.2	29.5 $\pm$ 5.4	0.261†
Diabetes mellitus duration (years)	§	11.9 $\pm$ 6.7	9.9 $\pm$ 7.6	15.8 $\pm$ 4.9	19.5 $\pm$ 16.7	0.178†
Fasting glucose level (mg/dL)	§	128.3 $\pm$ 42.6	131.2 $\pm$ 27.5	177.4 $\pm$ 71.2	169.3 $\pm$ 113.9	0.439†
Neuropathy degree score	0.63	1.0 $\pm$ 0.6	2.9 $\pm$ 0.9*	5.1 $\pm$ 0.5*	7.6 $\pm$ 1.9*	<0.001†

*Group statistically different from all others, Bonferroni pairwise comparisons.

†ANOVA.

‡ χ^2 test.

§No data.

familiarization tasks (knee flexion and extension) to achieve the requested force level with a visual feedback system. The assessments were made as follows. First, maximal voluntary contraction (MVC) was recorded twice (3-s with a 1-min pause) to determine the participant'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. Second, low-level force recordings of 6 isometric contractions (10-s each with 1-min pauses) of each task were performed, 2 contractions at 10% MVC, 2 contractions at 20% MVC, and 2 contractions at 30% MVC for knee flexion and extension. Visual feedback was given, and an error of $\pm 2\%$ 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 1 session.^{26,27} After the placement of the sEMG electrode matrix, the participants 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 with seat belts (Fig. 1).

High-Density sEMG and Isometric Force Recordings.

High-density sEMG from VL and BF muscles was acquired via matrices of 64 electrodes (ELSCH064NM2; OT Bioelettronica, Turin, Italy). The matrix consisted of 13 rows and 5 columns with one missing electrode (2-mm diameter, 8-mm interelectrode 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 and for BF, ischial tuberosity and lateral condyle of tibia. The matrices were attached to the skin by 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 mid-way portion. The sEMG signals were recorded monopolarly, amplified 1,000 times, sampled at 2,048 HZ (EMG-USB2+; OT Bioelettronica; 256-channel sEMG amplifier, -3dB

bandwidth 10–500 HZ), and digitized with a 12-bit A/D converter. Force signals were amplified 100 times, 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, 1,000 N range) customized in a dynamometer (OOR1; OT Bioelettronica). 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 in MATLAB R2016a (MathWorks, Natick, MA). Force data were downsampled to 100 HZ and low-pass filtered at 10 HZ (fourth order). For MVC trials, the mean force was computed during 500-ms windows with 100-ms overlap, and the highest value was considered as the peak force.

For force recordings at 10%, 20%, and 30% of MVC, the amount of force variability was quantified by the SD of the exerted force during the 5-s central epoch of the 10-s trial.²⁸ The force signal was inspected, and, if the mean force in the selected 5-s epoch was not in the $\pm 2\%$ range, a new 5-s window was selected for analysis. The selected window was maintained for all force and sEMG analyses. Non-linear analysis was also performed to assess the structure of force variability. The sample entropy (SaEn) of the exerted force was calculated during the same 5-s window 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.²⁹ The choice of the embedding dimension (m) and the tolerance distance (r) values used to define the space state and similarity criterion, respectively, are crucial. We set m to 2 and r to $0.2 \times SD$ of the force signal, in line with previous studies.^{28,30,31} SaEn is a unitless, nonnegative number with higher values indicating a less regular, unpredictable, and complex structure of the signal time series.³²

High-Density sEMG Data Analysis. The recorded VL and BF monopolar sEMG signals were off-line bandpass filtered (10–500 HZ, fourth-order Butterworth filter). A notch filter of 60 HZ and its harmonics (120, 180, 240, 300, 360, 420, and 480 HZ) were applied to diminish the interference of the power line. Fifty-nine bipolar sEMGs were obtained

FIGURE 2. Mean ($\pm$ SD) of the SD (N) and 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. Asterisks indicate statistical differences detected in *post hoc* tests. MVC, maximal voluntary contraction; SaEn, sample entropy.

along the columns of electrodes (12×5 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 during the same epoch used to compute the force level divided into 5 consecutive 1-s 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. Additionally, 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 ($m = 2$ and $r = 0.2 \times SD$) to characterize the complexity of the sEMG. SaEn was calculated during the same 5 consecutive 1-s epochs without overlap for each bipolar derivation. The mean of SaEn obtained from 5 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.²⁸

Statistical Analysis. Descriptive statistics are reported as mean (SD). Demographics, anthropometrics, and clinical variables were compared among groups by ANOVAs, followed by Bonferroni *post hoc* or χ^2 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%, and 30% of MVC) with the independent factor containing 5 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

FIGURE 3. Mean ($\pm$ SD) of the SD (N) and SaEn (unitless) 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. MVC, maximal voluntary contraction; SaEn, sample entropy.

group comparisons, and g values were considered small between 0.2 and 0.5, medium between 0.5 and 0.8, and large above 0.8.³⁸ The significance level was set at 5%, but because our study may be underpowered because of 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 SPSS Statistics 23.0 (IBM, Armonk, NY).

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; $F = 0.585$, $P = 0.675$).

ANOVA results, Hedge's effect sizes, and their corresponding CIs are shown in Supporting Information Table 1. MANOVA output showed a significant difference in the knee extension force during low-level contractions among groups (Wilk's $\Lambda = 0.500$, $F = 2.756$, $P < 0.001$). ANOVA showed a significant group effect on force SD at 10% ($F = 6.382$, $P < 0.001$), 20% ($F = 3.878$, $P = 0.006$), and 30% ($F = 5.392$, $P = 0.001$) of MVC tasks. Figure 2 shows the mean ($\pm$ 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

FIGURE 4. Mean ($\pm$ SD) of RMS (% MVC) and SaEn (unitless) 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. Asterisks indicate statistical differences detected in *post hoc* tests. MVC, maximal voluntary contraction; RMS, root mean square; SaEn, sample entropy.

control and absent groups. At 20% MVC, the moderate DPN group had significantly higher force SD than the control group, and at 30% MVC the moderate DPN group had higher force SD than control, absent, and mild DPN groups. 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 results of the *post hoc* comparisons and effect sizes. At 10% MVC the moderate DPN group had lower force SaEn than control and absent groups, and the severe DPN group presented lower force SaEn than the control group. At 20% MVC, absent and moderate groups showed significantly lower SaEn values than the control group.

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; $F = 0.765$, $P = 0.554$).

ANOVA results, Hedge's effect sizes, and their corresponding CIs are shown in Supporting Information Table 2. MANOVA output did not show a significant difference in knee flexion force during low-level contractions among groups (Wilk's $\Lambda = 0.689$, $F = 1.334$, $P < 0.140$). However, the effect sizes showed that the severe group presented higher force SaEn than the control group at 10% MVC and higher force SaEn than control, mild,

FIGURE 5. Mean ($\pm$ SD) of RMS (% MVC) and SaEn (unitless) 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. Asterisk indicates statistical difference detected in *post hoc* test. MVC, maximal voluntary contraction; RMS, root mean square; SaEn, sample entropy.

and moderate groups at 20% MVC (large effect sizes). Figure 3 shows the mean ($\pm$ SD) and effect sizes of the SD and SaEn during knee flexion.

Amplitude, Amount, and Structure of sEMG Variability During Knee Extension. Figure 4 shows the mean ($\pm$ SD), *post hoc* comparisons, and effect sizes of the RMS and SaEn of VL during knee extension. ANOVA results, Hedge's effect sizes, and their corresponding CIs are shown in Supporting Information Table 3. MANOVA output showed significant differences of sEMG variables among groups (Wilk's $\Lambda = 0.498$, $F = 2.774$, $P < 0.001$), and univariate ANOVA showed 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, severe individuals presented significantly lower SaEn compared with controls.

Amplitude, Amount, and Structure of sEMG Variability During Knee Flexion. Figure 5 shows the mean ($\pm$ SD), *post hoc* comparisons, and effect sizes of the RMS and SaEn of the BF during knee flexion. ANOVA results, Hedge's effect sizes, and their corresponding CIs are shown in Supporting Information Table 4. MANOVA output showed significant differences of sEMG variables among groups (Wilk's $\Lambda = 0.609$, $F = 1.806$, $P = 0.013$), and

univariate ANOVA showed significant group effect for BF SaEn at 10% MVC ($F = 2.977$, $P = 0.024$). *Post hoc* comparisons showed that severe participants had significantly lower BF SaEn than controls at 10% MVC.

DISCUSSION

Just as we had 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 diabetic individuals at all contraction levels.

All participants were able to maintain the required force levels for 10 s. 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,²⁸ is also an important aspect because a lower complexity of a time series (i.e., with lower structure) has been associated with disease.^{18,34}

During knee extension at 10% MVC, mild and moderate DPN participants demonstrated higher force SD than healthy participants, and moderate and severe DPN participants had both higher force SD and lower force SaEn than controls and participants without DPN. During knee extension at 20% MVC, moderate and absent DPN participants had higher force SD and lower force SaEn than controls. At 30% MVC moderate, DPN participants showed higher force SD than controls, mild and absent DPN participants. The presence of DPN, mainly starting from a moderate degree, affects the amount and structure of variability of force delineating a less adaptable system.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, for example, a less stable balance.^{36,37} Additionally, 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.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 participants might be detrimental during the performance of daily tasks because 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.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, whereas no major differences were found in the sEMG amplitude. All DPN groups (i.e., mild, moderate, and severe) showed lower VL SaEn compared with the matched control group, as had been reported by Golderberg *et al.*¹⁸ Additionally, at 20% and 30% MVC, only severe participants showed lower SaEn compared with controls. The structure of variability depicts how the system output changes over time.⁴⁰ Thus, the lower structure of variability in sEMG represents a less complex and unpredictable system characterized by a reduced functional capacity.⁴¹ Furthermore, the low SaEn values of VL activity in severe DPN participants most likely depict a lower ability to take advantage of the redundancy of the motor system. With the progression of DPN, the motor neurons can present signs of neuromuscular junction loss and axonal degeneration² that result in loss of motor units,³ characterizing a loss of functional components in the motor system that would lead to the observed low complexity in severe patients. Watanabe *et al.*⁴² 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 participants. 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 because it eccentrically controls the knee flexion during the loading phase.⁴³ Alterations in intralimb joint coordination during gait have been described in DPN participants, and a less variable pattern is observed in DPN participants in comparison with healthy participants.⁴⁴ 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 observed only in the effect sizes at 10% MVC, when the severe group presented a

higher SaEn than the control group, and at 20% MVC, when the severe group presented a higher SaEn than the control, mild, and moderate groups. Such inverse relationships had been reported earlier^{45,46} and can be explained by taking into consideration complexity tradeoffs between macroscopic (force or movement) and microscopic (sEMG) levels of a system.⁴⁷ 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 because of the fact that the participants reported difficulty in performing MVC knee flexions and discomfort. Another limitation of the study is the small sample size of the moderate and severe groups that could have masked the existence of additional alterations resulting from DPN, apart from what is described above.

Overall, the changes demonstrated in force and sEMG variability during knee extension show 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. Participants with moderate DPN showed a consistent pattern of alterations in that they presented a higher amount of force variability in all the studied force levels, whereas both moderate and severe participants presented a lower structure of force variability. Severe participants 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. Furthermore, the fact that most of the changes were observed at 10% MVC suggests the relevance of studying low-level contractions because the motor system has more latitude to adapt motor strategies. The force signal is influenced by the discharge variability of the active motor units,²¹ and DPN probably interferes with the recruitment of motor units and firing characteristics. Additionally, the fact that the participants with moderate DPN showed consistent findings might reflect that some of the more severe participants 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.³ The compensatory effects of the axonal sprouting are

considered limited,^{3,4} but they might have contributed, to some extent, to the apparent recovery presented in some of the variables in the severe DPN individuals. Coactivation strategies might also have been affected by the disease and its progression and interfered with motor control. Thus, future studies must also compute indexes as cocontraction or mutual information among muscle pairs^{48,49} in relation to DPN. Also, the coordination of synergistic muscles might be affected, given that there is evidence that synergic thigh muscles share synaptic input.⁵⁰ Besides that, type Ia muscle fibers may be dysfunctional in diabetic patients.⁵¹ Future studies recording sEMG activity from synergic muscles simultaneously and investigating the effects of DPN on coordination are warranted.

In conclusion, our findings reveal that DPN affects the complexity of the neuromuscular system at the knee for the extension task during low-level isometric contractions. The neuropathic participants with moderate and severe neuropathy presented the majority of the alterations showing the effects of DPN in the knee motor control. Our results provide insights for rehabilitation programs that should focus on restoring neuromuscular control of the knee with, for example, exercise therapy, strength training, or Taiji training.^{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.

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.

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6. GENERAL DISCUSSION

The hypothesis of this study is that DPN alters the motor complexity, expressed as an increased amount of variability and a decreased structure of variability in HD-sEMG and force outputs and that these alterations are aggravated by the severity of the disease. The main findings were that DPN increases the amount and decreases the structure of ankle and knee force variability, as expected, but the majority of the alterations were observed in moderate subjects. DPN did not affect the amount of muscular activity variability, but had an effect in the structure of its variability, and this effect occurred earlier or later in the disease progression, depending on the studied muscle. DPN decreased the structural variability earlier (mild stage) in TA and later (severe stage) in VL muscle. Furthermore, the diabetes, despite the presence of DPN, also affected the complexity of muscular activity, with diabetic subjects without DPN also showing alterations.

6.1 Motor complexity in the force output

DPN caused a reduction in the complexity of the force data, as shown by variability's both constructs – amount and structure. The amount of force variability was increased and the structure of force variability was reduced in the most affected subjects (moderate and severe-DPN groups).

To maintain a constant low level of force, the neuromuscular system has to be capable of perceiving very small force oscillations and correcting these oscillations, producing a steadier output. If the observed response in DPN subjects is an enhanced oscillation, then, motor control is affected. In addition, studies demonstrated that as the structural variability increases, the system adaptability also increases, and this capacity is important to respond to internal and external perturbations (Lipsitz, 2002). In the present study, the lower structure of force variability in DPN underlined the effect of the disease on motor control during isometric contractions.

Previous studies showed that the amount and structure of force variability during isometric contractions changes according to the level of force exerted. Increases in the force level induces increments in the amount of force variability, while the structure of force variability changes according to an inverted U-shaped function, with the top of the curve corresponding to the higher values of structural force variability, situated around 40% of MVC (Slifkin and Newell, 1999; Hong, Lee and Newell, 2007; Svendsen and Madeleine, 2010). The present study evaluated low-level forces to explore the effects of DPN in motor control during a task that would mainly recruit type I fibers (Duchateau and Enoka, 2011), which seem to be more affected by the disease. Besides the assessment of the isometric contractions at 10% of MVC, two additional force levels (20 and 30% MVC) were evaluated to investigate the changes in motor control at low contraction levels, in a range where the amount of variability should be increasing and the structure of variability should be decreasing with increments in the force. The additional force levels were used to verify if the recruitment patterns observed at 10% MVC would change with the presence of DPN,

or, in other words, if the recruitment strategy shown in the affected subjects would be reversed with the recruitment of additional motor units and/or the recruitment of different types of fibers with increments in the force level, according to Henneman's principle. If so, the differences between groups observed at 10% and/or 20% of MVC would no longer be observed at 30% MVC. Since the results changed according to the force level, the current study clearly showed that low-level contractions (below 30% MVC) cannot be considered as an entity and that the level of muscle activation greatly influences the measured effects of DPN on motor activities.

Amount of force variability

The amount of force variability, measured as force-SD, was increased in the most affected subjects (moderate and severe-DPN groups). Moderate subjects showed (i) higher force-SD than controls, absent and mild subjects at dorsiflexion at 10% and 20% MVC, (ii) higher force-SD than controls at plantarflexion at 30% MVC, (iii) higher force-SD than controls at knee extension in all studied levels, and higher than absents and milds at knee extension at 30% MVC. Severe subjects showed higher force-SD than controls, absent and mild subjects at dorsiflexion at 10% MVC and higher than controls at 20% MVC (shown as large effect size). For the knee extension, severe subjects also presented higher force-SD than controls at all force levels, as shown by the large effect sizes. Only during knee flexion, force-SD did not show a significant difference among groups. Altogether, these results demonstrate a clear effect of DPN in the amount of force variability.

Muscular force is produced by two main mechanisms – the recruitment of MUs and the modulation of active MUs firing rates of the active MUs, and the way in which these two mechanisms are elicited will determine how we interact with the environment (De Luca and Contessa, 2015). At low-level forces, MUs recruitment is the main process used for force increases (Sosnoff, Valantine and Newell, 2006), and as the recruitment order follows the size principle (Henneman, Somjen and Carpenter, 1965), less MUs and mainly type I fibers are recruited (Duchateau and Enoka, 2011). Increases in the force level causes increases in the force variability. This higher variability is mainly due to the recruitment of larger MUs that present a more phasic activity (Slifkin and Newell, 1999). Also, muscles with larger number of MU produce less force output variability (Hamilton, Jones and Wolpert, 2004; Sosnoff and Newell, 2006). If DPN leads to a loss of MUs, particularly the smaller ones, than force variability during the performance of isometric contractions would be higher.

Evidences that DPN causes a loss of MUs have previously been described (Souayah *et al.*, 2009; Allen *et al.*, 2013; Allen *et al.*, 2014a) and could indeed be contributing to the augmented force variability. Dideriksen *et al.* (2012) also suggest that decreases in the force steadiness might be related to reductions in the size of MU populations. Furthermore, ankle muscles of DPN subjects, especially TA, present loss of muscle mass and strength (Andersen, 1999; Andersen *et al.*, 2004; Moore *et al.*, 2015) and would cause a higher force output variability while producing torque. Although the studies mostly report distal muscles as showing the majority of neuromuscular alterations due to DPN, it is not surprising that knee extension also presented a higher force-SD in the most compromised subjects. There are evidences of motor axon losses

in VL muscles (Andersen *et al.*, 1998) and a series of biopsy studies have shown a decrease of type I muscular fibers in the VL muscle of diabetic patients (Marin *et al.*, 1994; Gaster *et al.*, 2001; Oberbach *et al.*, 2006; Mogensen *et al.*, 2007; Larsen *et al.*, 2009). If type II muscle fibers, that present a more phasic activity, are mainly being recruited, more variability should be expected in the force output. It is also important to call attention for the fact that DPN, despite its proximal to distal evolution, is also affecting the motor control of more proximal joints.

Structure of force variability

Subjects with DPN presented lower structure of force variability, following a similar pattern to what we observed in the amount of force variability, but in an inverted order, i.e., lower level of structural variability instead of the higher amount of variability. During dorsiflexion, moderate DPN group showed lower force structural variability in all studied force levels, while during plantarflexion structural force variability was reduced at only 30% MVC in all diabetic groups (absent, mild, moderate and severe). During knee extension at 10% MVC, both moderate and severe groups showed lower force structural variability, while at 20% MVC, only moderate group showed this decrease. Interestingly, at 20% MVC the absent group also showed a reduced force structural variability when compared to the control group.

While the SD provides an index of the degree of deviation from a point, representing the magnitude of fluctuations in the system output, the SaEn measures the variation in the system output along another dimension – time – that is independent of the magnitude. More specifically, SaEn measures the way the system

output changes over time, which represents the structure of the system dynamics (Slifkin and Newell, 1999). Decreases in the structure of force variability (force-SaEn) might occur due to the decrease in the number of process components that are contributing to the system output. As mentioned before, force levels are adjusted by using two different neural control strategies: the recruitment of active MUs and the modulation of discharge frequencies of already active MUs. Slifkin & Newell (Slifkin and Newell, 1999) have shown that the complexity of the force output changes as an inverted U-shaped function over the range of force levels, meaning that at the lower and higher force levels, the complexity is low, and at some point between this two extremities, the complexity is high. The region of higher complexity is located at about 40% MVC. To achieve low force levels, force generation is mainly due to the recruitment of MUs, and to achieve higher force levels, force generation and maintenance depend on the modulation of discharge frequencies. In the range where force complexity is maximum, which would be around 40% of MVC, both mechanisms are active, meaning that a larger number of process components are contributing to system output. Higher complexity of a signal output suggests that more information is being transmitted from the force signal, as a consequence of the presence of both physiological mechanisms. Therefore, the observed reduction of the structural force variability in the presence of DPN might also have occurred due to the loss of MUs. If fewer MUs are available, the response will be more stereotyped, and the force output will be more regular along time, then lower structural variability will be observed, exactly as we did.

During knee flexion, the severe group presented a higher SaEn than the control group at 10%, and the severe group presented a higher SaEn than the controls, mild and moderate groups at 20% MVC, showing a tendency of higher structural variability in the severe group. Such inverse relationship of a higher complexity in a more severe stage of the disease, have been reported earlier (Morrison, Hong and Newell, 2007; Rathleff *et al.*, 2011) and can be explained by taking into consideration complexity tradeoffs between macroscopic (force or movement) and microscopic (sEMG) levels of a system (Bar-Yam, 2004). Torque, in the other hand, is a more general and gross measure, resulting from both agonists and synergistic muscles contractions, being also affected by other factors besides muscle activity, such as joint stiffness, lever arm and dynamic interactions with the environment, as the equipment, for instance. Duarte and Sternad (2008) also reported a higher complexity in the center of pressure measurements during prolonged standing in older adults, which would be expected to show lower complexity, in comparison to young ones. One of the author's justifications is that older subjects might have more difficulty to adapt to the environmental demand, which would result in a higher complexity. The most severe subjects might have had problems in generating the optimal force output and, thus, variability, due to the difficulty of the task, responding with a higher structural variability without increases in the amount of variability.

6.2 Motor complexity in the muscular activation patterns

DPN reduced the complexity of the muscle activity represented by a reduced

structural variability in the sEMG signal of TA and VL muscles. The fact that the level of muscle activation was not affected by the disease shows that the neuropathic subjects are still able to produce the amount of activity that is necessary to meet the task demand. But the observed changes in the structural variability clearly show that motor control is degraded in these subjects. The controversial results of the sEMG studies during dynamic tasks with DPN population can be partially explained by the supposed motor control degradation and complexity alterations that are not easily extracted from bipolar electrodes and discrete/ linear analysis, which are the usual method used in these previous studies (Abboud, Rowley and Newton, 2000; Kwon *et al.*, 2003; Sacco and Amadio, 2003; Akashi *et al.*, 2008; Sacco, Akashi and Hennig, 2010; Savelberg *et al.*, 2010; Gomes *et al.*, 2011; Sawacha, Spolaor, *et al.*, 2012; Watari *et al.*, 2014).

Level of muscular activation

We did not observe a higher amount of variability of the sEMG in the muscles from DPN subjects, as hypothesized. The amount of variability of the sEMG, measured as the RMS value, corresponds to the level of muscular activation and the only observed alterations were for the TA muscle from diabetic subjects without DPN.

The absent DPN group showed higher TA activity (RMS) when compared to healthy subjects at 10% and 20%MVC. This result shows that diabetes, despite the presence of DPN, plays a key role in the performance of the TA. Higher sEMG signals amplitudes are related to the recruitment of additional MUs and possibly changes in the firing properties of the active MUs (Duchateau and Enoka, 2011). The clinical progression of diabetes causes alterations in the structure and composition of muscle

proteins (Bozkurt, Severcan and Severcan, 2010), compromising the muscle quality, defined as the strength per unit mass, and the contractile quality, already described in the TA muscle of diabetic patients (Park *et al.*, 2006; Ijzerman *et al.*, 2011; Moore *et al.*, 2015; Guerrero *et al.*, 2016). Thus, increased sEMG amplitude could represent an initial compensatory mechanism that occurs in response to structural changes in the muscles of diabetic subjects. The fact that DPN subjects did not show the alterations presented in diabetic subjects without DPN anymore might be because of a loss of this compensatory mechanism due to DPN effects and the use of other compensatory mechanisms. Since alterations were found in the structural variability of TA sEMG, alterations in the recruitment patterns are present, but the adopted strategies are not producing increases in the level of muscular activity.

The fact that only TA showed a difference in the level of muscular activation might be because it is smaller than the other evaluated muscles, and, therefore, the loss of muscle mass that happens due to diabetes (Guerrero *et al.*, 2016) might have had a major effect in it. Differences in fiber type composition can also explain this result, since diabetes seems to affect more type I muscle fibers (Marin *et al.*, 1994; Gaster *et al.*, 2001; Oberbach *et al.*, 2006; Mogensen *et al.*, 2007; Larsen *et al.*, 2009).

Structure of muscular activation variability

DPN reduced the structure of sEMG variability of TA and VL activities. At 10%MVC, TA showed lower structure of variability of muscle activity pattern in all diabetic groups compared to control group, regardless the presence or severity of the DPN. With an increase in the force level (20 and 30% of MVCs), this difference was

more evident between healthy subjects and those with mild DPN. We also observed by the greater effect sizes a distance in the patterns found in the controls and in all other diabetic groups (absent, mild, moderate and severe), where the diabetic subjects presented a lower structure of variability of muscle activity. Altogether, these results show a clear effect of both diabetes and DPN, regardless its severity, meaning that the alterations in the complexity of muscular activity occur in the very early stages of diabetes and DPN.

Allen *et al.* (2015) evaluated the intramuscular activity of TA in DPN subjects during isometric contractions at 25% MVC. These authors have also showed that DPN individuals had higher activity but fewer MUs activated when compared to controls. According to concepts of physiological complexity (Goldberger, Peng and Lipsitz, 2002; Lipsitz, 2002), if reduced degrees of freedom of the motor system are available (fewer MUs), a less complex signal output would be generated, as observed in the present study.

The effect sizes for the pairwise comparisons showed a trend for the same pattern observed in the TA for the VL muscle, with all diabetic groups showing lower sEMG structural variability in comparison to the control group. Additionally, a more pronounced effect of DPN can be seen in the VL. DPN diminished the complexity in VL sEMG, particularly at 10%MVC, with all DPN groups (i.e. mild, moderate and severe) presenting lower VL SaEn when compared to the controls. The structure of variability depicts how the system output changes over time (Slifkin and Newell, 1999). Thus, the lower structure of variability in sEMG represents a less complex and unpredictable system characterized by a reduced functional capacity (Lipsitz, 2002). Further, at 20%

and 30% of MVC only severe subjects showed lower SaEn compared with controls, showing that at the latest stage of DPN depict a lower ability to take benefit from the redundancy of the motor system. With the progression of DPN, the motor neurons can present signs of neuromuscular junction loss and axonal degeneration (Zochodne, Ramji and Toth, 2008), which result in a loss of MUs (Ramji *et al.*, 2007), characterizing a loss of functional components in the motor system that would lead to the observed low complexity in severe patients.

Watanabe *et al.* (2012) demonstrated that individuals with type-2 diabetes mellitus have a limited area of activation within VL muscle with an attenuated spatial pattern of sEMG compared with age-matched control subjects. The HD-sEMG was measured during isometric knee extension at 10% of 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 a result of a limited number of MUs recruited to perform knee extension. Thereby, loss of MUs due to DPN seems to play an important role in the reduction of VL complexity.

The TA and VL results for sEMG complexity show that the TA is affected for both effects of diabetes and DPN in the muscle activity, while VL is more affected by DPN. It is also interesting to note that the effects of DPN seem to appear earlier in TA during the course of the disease, since mild-DPN subjects already show differences in all studied force levels. For VL, these differences are more pronounced in the later stages, if we take the results of all three force levels together. It also calls attention the differences observed between the patterns showing at 10% of MVC and at 20%-30% of MVC, that remained the same, regardless the muscle – TA or VL. At the lowest force

level (10% of MVC), fewer MUs are recruited, and with increments in the force, additional and larger MUs are recruited. Therefore, if mild and moderate subjects show lower structural variability in VL only at 10% of MVC, at 20% and 30% of MVC the losses of the smallest MUs are compensated by the recruitment of larger MUs, making it possible to maintain the amount of information that is transmitted during the muscle activation. In the severe subjects, this capacity is lost, and one can infer that the progressive MUS degeneration would be a major cause. On the other side, TA muscle already presents lower structural variability in the absence of clinical DPN, showing that MUs dysfunction might already be present in subclinical levels, as it has already been shown previously (Malik *et al.*, 2005; Meijer *et al.*, 2008; Sung *et al.*, 2012) and, maybe, TA muscle is a more susceptible muscle.

The fact that mild subjects already show alterations at 20% and 30% of MVC in TA muscle, in contrast to what happens in VL, demonstrates an earlier TA impairment in the course of the disease. Two reasons might explain for this difference between muscles: (i) the fact that TA is a more distal muscle, and expected to be more affected, since the progression of DPN is distal to proximal; (ii) TA might also be more susceptible to the effects of diabetes itself, despite of DPN, like loss of muscle mass and deterioration of contractile quality due to differences in muscle size and fiber type composition (Andersen *et al.*, 1997; Andersen, Gjerstad and Jakobsen, 2004; Park *et al.*, 2006; Ijzerman *et al.*, 2011; Allen *et al.*, 2014b; Moore *et al.*, 2015; Guerrero *et al.*, 2016). As previously presented and discussed, TA was also the only muscle that showed alterations in the level of muscular activation, with absent group showing higher muscular activation in comparison with control group.

As unexpected, at 10 and 20% of MVCs, the GM sEMG data of severe patients showed more complexity when compared to control and moderate groups. If we look only at the data from control group, the complexity of GM muscle activation decreased as the force level increased. In an opposite direction, severe subjects showed a trend for a higher muscle activity complexity at 10%MVC. If severe subjects present a higher SaEn to perform a task, that is easy to perform, it could be interpreted as they are making adjustments to compensate for the sensorial deficits due to the DPN. Thereby, the adjustments would reflect in an increase in the complexity of muscle activity, suggesting that severe subjects are using more resources from the motor system than necessary in a normal condition to accomplish the task.

The reduction or absence of ankle reflexes have been described in patients with DPN (Boulton, 2005). Although the ankle reflex was not evaluated in our participants, impairments are probably present in the most severe subjects. These impairments would affect the sensorial input and alter proprioception that would affect the recruitment strategies of GM muscle. As the force level increases, the recruitment pattern would need to change in order to perform these adjustments. However, this change would not be possible in the case of the most severe subjects due to the losses caused by the progressive denervation and this might explain why the severe subjects did not maintained a pattern of GM higher complexity at higher forces when compared to control subjects. We also observed a higher force structural variability during knee flexion in severe subjects, as previously discussed, showing that increases in motor complexity can be a response to the disease status.

For the BF, the only significant difference observed in the SaEn at 10%MVC among groups was that the severe group showed a lower complexity than controls. It can be argued that the isometric contraction during the knee flexion performed in the study setup (sitting position and knee flexion at 45°) did not recruit enough MUs allowing detection of differences among 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 discomfort and difficulty in performing MVC during knee flexion.

6.3 Motor complexity – General considerations

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 (Svendsen and Madeleine, 2010), is also an important aspect since a lower complexity of a time series (i.e. with lower structure) has been associated with disease (Stergiou, Harbourne and Cavanaugh, 2006) and DPN clearly decreases structural variability of force and muscle activity.

A novel and important finding was that motor variability seemed to be more affected in moderate diabetic subjects, especially at the ankle joint, while severe cases showed an apparent recovery of motor-control strategies.

As DPN is progressive, the observed pattern of alteration suggests that in the moderate stage, where the DPN is certainly installed, the effects of this disease on motor variability are more apparent. Similar findings have been reported regarding

pain. Increases in motor variability were found in acute stages of pain, while during subchronic and/or chronic stages of pain, motor variability decreased (Madeleine, Mathiassen and Arendt-Nielsen, 2008; Madeleine, 2010), as we observed in a more chronic stage of DPN – moderate group. However, severe subjects, who represent the later stage of DPN, did not maintain the same pattern of alterations for some of the studied variables. One of the responses to the denervation caused by DPN is the axonal sprouting, which allows the reinnervation of muscle fibers by intact neighboring MUs (Ramji *et al.*, 2007). Although studies have provided evidence that the compensatory effects of axonal sprouting are limited (Ramji *et al.*, 2007; Allen *et al.*, 2013), such effects might have contributed to the recovery in the amount and structure of force variability in the severe group. The fact that this altered pattern is more observed at the ankle level, that seems to be more severely affected, and in an earlier stage of the disease, also suggests that the compensatory mechanisms, as axonal sprouting, might be present with larger magnitude and contribute more for this apparent recover of ankle motor control in severe subjects.

Furthermore, the system used to classify the severity of DPN took into account signs and symptoms related to the sensorial aspects of DPN. Nevertheless, although the fuzzy system has been shown to be highly accurate when classifying the severity of DPN patients (Watari *et al.*, 2014), motor alterations may not follow the same path as the sensorial alterations. The classification system adopted may explain the failure to find a progressive effect of DPN according to the severity of the disease. Additionally, the small sample size of the moderate and severe groups could have masked the existence of other alterations due to DPN, although it was possible to detect some

significant differences between the studied groups.

Coordination between individual muscles might be affected by DPN, although we cannot infer that with our results, since we only evaluated one agonist muscle per task. Laine *et al.* (2015) showed that there is a high common drive between vasti muscles, showing the presence of a cross-muscle drive between synergists and suggest that this common drive contains both Ia feedback and a descending cortical component. Morphological studies in DPN do not describe alterations in Ia fibers, but diabetes compromises the microcirculation and has a systemic effect, so it could be affecting the Ia fibers function. Van Deursen *et al.* (1998) have described evidences that DPN degrades the muscle spindle function. De Luca *et al.* (2009) also showed that the proprioceptive feedback from spindles interferes with the common drive. Thus, future studies should take the common drive into consideration when exploring motor control alterations due to DPN.

There is still a need for a deeper understanding about the effects of DPN in MUs recruitment patterns. It has been shown that during voluntary constant-force contractions, earlier-recruited MUs maintain higher firing rates than later-recruited ones, and this phenomenon occurs at any time and force level. It has been described as the Onion-Skin scheme, because when the firing rates of the MUs are plotted as functions of time, they assume the same layer pattern observed in an onion (De Luca and Erim, 1994; De Luca and Hostage, 2010). This scheme would allow producing force more quickly and more smoothly when force is initiated, and would enhance the capacity of sustain force over longer time (De Luca and Contessa, 2015). If DPN causes loss of MUs, which kind of adaptations in the recruitment patterns have to be made in

order to compensate for this loss? If smaller MUs are lost in the progression of the disease, how this Onion-Skin scheme would work? The fact that in general the amount of activation did not change in the studied groups and muscles, but the structural variability changed in presence of DPN, might suggest the existence of alterations in this recruitment pattern. Studies with MUs decomposition techniques would also add to the understanding of motor control alterations present in DPN.

6.4 Clinical implications

Ankle and knee movements are very important for locomotor activities, such as walking. Furthermore, TA and VL muscles, that showed the majority of alterations in this study, have important functions during locomotor activities. TA is an important muscle that controls the ankle extension at the early stance via eccentric contraction and VL is responsible for controlling eccentrically the knee flexion during the loading phase (Winter, 1991). Thus, it is conceivable that the observed alterations in the variability of the force and muscle signals would compromise daily living activities in the presence of DPN.

Locomotor tasks in environments with different mechanical demands will require motor strategies to adapt to them. The observed degraded complexity will affect the choice and implementation of these strategies. The DPN is already a condition that compromises the sensorial system that will be affecting the relationship between the neuromuscular system and the environment.

Alterations of the ankle control during gait have already been described in DPN subjects, which showed a less extended ankle and a smaller ankle extensor moment at push-off (Sacco *et al.*, 2015). Moreover, alterations in the intra-limb joint coordination during gait have been described in DPN subjects, and a less variable movement pattern was observed in these subjects in comparison to healthy subjects (Yi *et al.*, 2016). This lower variability is observed at the ankle-knee and knee-hip continuous relative phase at early stance phase, when the knee contributes eccentrically to the load attenuation, and in the knee-hip relative phase at terminal swing phase, when the knee assumes the important task of propelling the limb. In our study, we showed that the motor complexity of ankle flexor and knee extensor muscles are already affected during very simple tasks, as isometric contractions. Thereby, the motor control during dynamic activities should also be compromised and being reflected in the abovementioned gait alterations of DPN subjects. Therefore, special attention should be addressed to the ankle flexors muscles, knee extensor muscles and synergistic muscle actions in the rehabilitation of DPN patients.

The amount of torque variability during isometric contractions has been shown to be correlated with motor performance, with larger plantarflexor torque variability being associated with e.g. a less stable balance (Mello, Magalhães and Kohn, 2013; Watanabe *et al.*, 2013). Further, practicing isometric plantarflexion contractions within 20% of MVC has been shown to diminish the amount of plantarflexion torque variability and improve the postural stability (Oshita and Yano, 2011). In addition, the greater amount of variability in the knee extensor torque would affect motor control 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 (Tikkanen *et al.*, 2013).

It has been shown that six-weeks of knee strengthening program (but not endurance training) reduced MUs discharge rate variability of vasti muscles and enhances the force fluctuations of the knee extensor during low-level contractions (Vila-Cha and Falla, 2015) and, thus, diabetic patients might have advantages in reducing variability of daily living tasks taking part in this type of training. Therefore, enhancing the motor control during the performance of isometric contractions of both ankle and knee joints at low-level forces via strengthening programs with and without feedback support will probably benefit DPN patients and may be taken into consideration during rehabilitation.

A 20-week protocol of Taiji training, which involves movements of the body aiming at enhance balance and stability, has also been shown to reduce knee extensor force SD, independent of improvements in strength (Christou, Yang and Rosengren, 2003). Therefore, rehabilitation protocols for DPN should also include the practice of tasks that challenge the ankle and knee motion control to stimulate the neuromuscular system to create new motor solutions. Functional exercises that uses changes of direction, cutting manoeuvres, presence of obstacles, tasks not performed in a daily-basis (e. g. walking backward), standing in uneven surfaces, diminishing the base of support, can be beneficial for the former purpose discussed. The practice of functional exercises within the rehabilitation protocol will also help to incorporate the gains obtained during the training of static contractions to the dynamic conditions, bringing

improvements to the synergistic muscles actions that are probably affected in DPN patients.

Although most of the alterations occurred at moderate and severe stages, alterations in force and sEMG complexities were present already in the mild subjects, and then the aforementioned interventions should be added in the early stages of DPN patients.

7. CONCLUSIONS

The main findings showed that DPN affects the complexity of the neuromuscular system during isometric contractions of the ankle and knee joints, reducing the capacity to comply with mechanical demands of low-level forces. The studied patterns showed higher amount and lower structure of force variability at dorsiflexion, plantarflexion, and knee extension, and lower structure of TA and VL sEMG variability.

The observed alterations were not associated with the DPN severity, and diabetic subjects with moderate DPN showed the majority of complexity alterations. Thus, diabetes alone also affected motor control, regardless of the presence of DPN.

Our results also provide insights for rehabilitation programs that should focus on restoring neuromuscular control of the ankle and knee at low-level contractions, strengthening protocols and including tasks that challenge the lower limbs motor control and allow the individual creating new biomechanical solutions. Interventions should also be planned in early stages of DPN, since alterations are already observed in the early stages of the disease.

ANNEX 1 - SUPPLEMENTARY MATERIAL FROM PAPER 1

Table S1 – Mean (SD) of force standard deviation (SD) and force sample entropy (SaEn) values during dorsiflexion for controls and neuropathy groups. F and P values for ANOVA tests. Hedges' effect sizes for comparisons between groups.

FORCE VARIABLE	CONTROL (1)	ABSENT (2)	MILD (3)	MODERATE (4)	SEVERE (5)	P	F	η^2_p	Hedges' g effect size	95% Confidence interval (g)	
										Inferior limit	Superior limit
Dorsiflexion SD at 10%MVC (N)	1.57 (1.16)	1.74 (1.03)	1.46 (0.89)	3.07 (2.20)	2.97 (1.74)	0.001	5.41	0.196	(1)x(2) = 0.156	-0.438	0.75
									(1)x(3) = -0.107	-0.713	0.499
									(1)x(4) = 0.902	0.186	1.618
									(1)x(5) = 0.983	0.261	1.705
									(2)x(3) = -0.29	-0.871	0.292
									(2)x(4) = 0.854	0.167	1.541
									(2)x(5) = 0.924	0.233	1.615
									(3)x(4) = 1.053	0.34	1.765
									(3)x(5) = 1.177	0.454	1.9
									(4)x(5) = -0.05	-0.791	0.691
Dorsiflexion SD at 20%MVC (N)	1.77 (1.14)	2.50 (1.22)	2.32 (1.44)	3.83 (1.84)	2.98 (1.61)	0.001	4.820	0.178	(1)x(2) = 0.616	0.009	1.224
									(1)x(3) = 0.421	-0.191	1.033
									(1)x(4) = 1.406	0.646	2.166
									(1)x(5) = 0.896	0.18	1.611
									(2)x(3) = -0.135	-0.715	0.444
									(2)x(4) = 0.902	0.212	1.592
									(2)x(5) = 0.349	-0.314	1.013
									(3)x(4) = 0.941	0.236	1.645
									(3)x(5) = 0.438	-0.24	1.116
									(4)x(5) = -0.492	-1.244	0.26
Dorsiflexion SD at 30%MVC (N)	3.22 (1.98)	4.00 (2.59)	3.49 (1.30)	5.16 (3.26)	3.76 (1.55)	0.132	1.818	0.076	(1)x(2) = 0.333	-0.264	0.931
									(1)x(3) = 0.163	-0.444	0.769
									(1)x(4) = 0.753	0.047	1.459
									(1)x(5) = 0.294	-0.392	0.981
									(2)x(3) = -0.244	-0.825	0.336
									(2)x(4) = 0.408	-0.258	1.073
									(2)x(5) = -0.107	-0.767	0.552
									(3)x(4) = 0.739	0.047	1.43
									(3)x(5) = 0.189	-0.483	0.86
									(4)x(5) = 0.551	-1.305	0.204

To be continued

Table S1 – Mean (SD) of force standard deviation (SD) and force sample entropy (SaEn) values during dorsiflexion for controls and neuropathy groups. F and P values for ANOVA tests. Hedges' effect sizes for comparisons between groups (conclusion).

FORCE VARIABLE	CONTROL (1)	ABSENT (2)	MILD (3)	MODERATE (4)	SEVERE (5)	P	F	η^2_p	Hedges' g effect size	95% Confidence interval (g)	
										Inferior limit	Superior limit
Dorsiflexion SaEn at 10%MVC (a. u.)	0.14 (0.07)	0.10 (0.03)	0.12 (0.04)	0.08 (0.03)	0.09 (0.04)	0.003	4.339	0.163	(1)x(2) = -0.653	-1.262	-0.044
									(1)x(3) = -0.335	-0.944	0.275
									(1)x(4) = -1.07	-1.799	-0.341
									(1)x(5) = -0.758	-1.464	-0.051
									(2)x(3) = 0.412	-0.172	0.997
									(2)x(4) = -0.745	-1.425	-0.065
									(2)x(5) = -0.258	-0.919	0.404
									(3)x(4) = -1.105	-1.822	-0.388
									(3)x(5) = -0.626	-1.311	0.06
Dorsiflexion SaEn at 20%MVC (a. u.)	0.12 (0.04)	0.095 (0.04)	0.11 (0.05)	0.07 (0.02)	0.098 (0.05)	0.028	2.852	0.114	(4)x(5) = 0.437	-0.312	1.187
									(1)x(2) = -0.547	-1.151	0.058
									(1)x(3) = -0.159	-0.765	0.448
									(1)x(4) = -1.305	-2.055	-0.555
									(1)x(5) = -0.407	-1.097	0.283
									(2)x(3) = 0.325	-0.257	0.907
									(2)x(4) = -0.749	-1.43	-0.069
									(2)x(5) = 0.065	-0.594	0.725
									(3)x(4) = -0.935	-1.639	-0.231
Dorsiflexion SaEn at 30%MVC (a. u.)	0.09 (0.03)	0.10 (0.03)	0.09 (0.03)	0.07 (0.02)	0.10 (0.05)	0.037	2.680	0.108	(3)x(5) = -0.226	-0.898	0.446
									(4)x(5) = 0.687	-0.075	1.45
									(1)x(2) = 0.328	-0.269	0.926
									(1)x(3) = -0.111	-0.717	0.495
									(1)x(4) = -0.915	-1.632	-0.198
									(1)x(5) = 0.171	-0.513	0.855
									(2)x(3) = -0.44	-1.025	0.146
									(2)x(4) = -1.442	-2.177	-0.708
									(2)x(5) = -0.062	-0.721	0.598
									(3)x(4) = -0.749	-1.442	-0.057
									(3)x(5) = 0.256	-0.416	0.929
									(4)x(5) = 0.812	0.041	1.582

Table S2 – Mean (SD) of force standard deviation (SD) and force sample entropy (SaEn) values during plantarflexion for controls and neuropathy groups. F and P values for ANOVA tests. Hedges' effect sizes for comparisons between groups.

FORCE VARIABLE	CONTROL (1)	ABSENT (2)	MILD (3)	MODERATE (4)	SEVERE (5)	P	F	η^2_p	Hedges' g effect size	95% Confidence interval (g)	
										Inferior limit	Superior limit
Plantarflexion SD at 10%MVC (N)	1.74 (0.99)	1.96 (1.05)	1.94 (0.69)	2.25 (1.31)	1.98 (0.98)	0.730	0.508	0.022	(1)x(2) = 0.219	-0.376	0.814
									(1)x(3) = 0.242	-0.366	0.85
									(1)x(4) = 0.452	-0.239	1.143
									(1)x(5) = 0.245	-0.44	0.931
									(2)x(3) = -0.021	-0.6	0.557
									(2)x(4) = 0.25	-0.412	0.911
									(2)x(5) = 0.017	-0.642	0.676
									(3)x(4) = 0.314	-0.36	0.988
									(3)x(5) = 0.045	-0.625	0.715
									(4)x(5) = -0.234	-0.977	0.51
Plantarflexion SD at 20%MVC (N)	1.78 (0.98)	2.26 (1.23)	2.25 (0.79)	2.53 (1.15)	1.99 (1.095)	0.305	1.228	0.052	(1)x(2) = 0.43	-0.17	1.03
									(1)x(3) = 0.54	-0.077	1.156
									(1)x(4) = 0.713	0.009	1.416
									(1)x(5) = 0.205	-0.48	0.89
									(2)x(3) = -0.004	-0.583	0.574
									(2)x(4) = 0.223	-0.438	0.884
									(2)x(5) = -0.23	-0.891	0.431
									(3)x(4) = 0.288	-0.385	0.962
									(3)x(5) = -0.291	-0.964	0.383
									(4)x(5) = -0.48	-1.232	0.271
Plantarflexion SD at 30%MVC (N)	1.93 (1.18)	2.89 (1.45)	2.95 (1.30)	3.34 (1.06)	2.68 (1.46)	0.033	2.742	0.110	(1)x(2) = 0.72	0.108	1.332
									(1)x(3) = 0.818	0.187	1.448
									(1)x(4) = 1.253	0.508	1.998
									(1)x(5) = 0.58	-0.116	1.277
									(2)x(3) = 0.04	-0.538	0.619
									(2)x(4) = 0.344	-0.32	1.007
									(2)x(5) = -0.142	-0.802	0.518
									(3)x(4) = 0.329	-0.345	1.003
									(3)x(5) = -0.193	-0.864	0.479
									(4)x(5) = -0.519	-1.273	0.234

To be continued

Table S2 – Mean (SD) of force standard deviation (SD) and force sample entropy (SaEn) values during plantarflexion for controls and neuropathy groups. F and P values for ANOVA tests. Hedges' effect sizes for comparisons between groups (conclusion).

FORCE VARIABLE	CONTROL (1)	ABSENT (2)	MILD (3)	MODERATE (4)	SEVERE (5)	P	F	η^2_p	Hedges' g effect size	95% Confidence interval (g)	
										Inferior limit	Superior limit
Plantarflexion SaEn at 10%MVC (a. u.)	0.12 (0.07)	0.10 (0.04)	0.08 (0.03)	0.10 (0.05)	0.10 (0.045)	0.271	1.315	0.056	(1)x(2) = -0.287	-0.884	0.309
									(1)x(3) = -0.653	-1.275	-0.032
									(1)x(4) = -0.233	-0.918	0.453
									(1)x(5) = -0.26	-0.946	0.425
									(2)x(3) = -0.497	-1.085	0.09
									(2)x(4) = 0.025	-0.634	0.684
									(2)x(5) = 0.005	-0.654	0.664
									(3)x(4) = 0.464	-0.214	1.143
									(3)x(5) = 0.492	-0.187	1.172
									(4)x(5) = -0.019	-0.76	0.722
Plantarflexion SaEn at 20%MVC (a. u.)	0.11 (0.04)	0.10 (0.04)	0.097 (0.05)	0.08 (0.04)	0.12 (0.05)	0.177	1.615	0.068	(1)x(2) = -0.262	-0.858	0.334
									(1)x(3) = -0.319	-0.928	0.29
									(1)x(4) = -0.836	-1.547	-0.125
									(1)x(5) = 0.06	-0.623	0.743
									(2)x(3) = -0.109	-0.688	0.47
									(2)x(4) = -0.667	-1.343	0.009
									(2)x(5) = 0.313	-0.35	0.976
									(3)x(4) = -0.432	-1.109	0.246
									(3)x(5) = 0.353	-0.322	1.028
									(4)x(5) = 0.828	0.056	1.6
Plantarflexion SaEn at 30%MVC (a. u.)	0.14 (0.07)	0.09 (0.03)	0.09 (0.05)	0.07 (0.03)	0.08 (0.03)	0.001	5.040	0.185	(1)x(2) = -0.877	-1.498	-0.256
									(1)x(3) = -0.733	-1.359	-0.107
									(1)x(4) = -1.066	-1.794	-0.337
									(1)x(5) = -0.911	-1.628	-0.195
									(2)x(3) = 0.054	-0.525	0.632
									(2)x(4) = -0.561	-1.232	0.11
									(2)x(5) = -0.241	-0.902	0.42
									(3)x(4) = -0.444	-1.122	0.234
									(3)x(5) = -0.217	-0.889	0.455
									(4)x(5) = 0.327	-0.418	1.073

Table S3 – Mean (SD) of root mean square (RMS) and sEMG sample entropy (SaEn) values from tibialis anterior muscle for controls and neuropathy groups. F and P values for ANOVA tests. Hedges' effect sizes for comparisons between groups.

SEMG VARIABLE	CONTROL (1)	ABSENT (2)	MILD (3)	MODERATE (4)	SEVERE (5)	P	F	η^2_p	Hedges' g effect size	95% Confidence interval (g)	
										Inferior limit	Superior limit
Tibialis anterior RMS at 10% MVC (%MVC)	1.79 (0.96)	3.58 (2.48)	2.53 (1.71)	2.50 (1.73)	3.01 (1.92)	0.034	2.729	0.109	(1)x(2) = 0.92	0.296	1.543
									(1)x(3) = 0.527	-0.089	1.143
									(1)x(4) = 0.535	-0.16	1.229
									(1)x(5) = 0.853	0.141	1.566
									(2)x(3) = -0.489	-1.076	0.098
									(2)x(4) = -0.482	-1.151	0.186
									(2)x(5) = -0.249	-0.91	0.413
									(3)x(4) = -0.017	-0.688	0.653
									(3)x(5) = 0.268	-0.405	0.941
Tibialis anterior RMS at 20% MVC (%MVC)	2.36 (1.22)	5.16 (4.88)	3.26 (1.24)	3.48 (1.67)	3.84 (2.80)	0.033	2.754	0.110	(4)x(5) = 0.279	-0.465	1.023
									(1)x(2) = 0.756	0.142	1.37
									(1)x(3) = 0.731	0.106	1.357
									(1)x(4) = 0.789	0.08	1.497
									(1)x(5) = 0.734	0.029	1.439
									(2)x(3) = -0.523	-1.112	0.065
									(2)x(4) = -0.417	-1.083	0.249
									(2)x(5) = -0.311	-0.974	0.352
									(3)x(4) = 0.155	-0.516	0.826
Tibialis anterior RMS at 30% MVC (%MVC)	3.57 (1.92)	5.75 (4.70)	5.36 (3.33)	3.63 (1.78)	3.84 (1.89)	0.081	2.154	0.088	(3)x(5) = -0.292	-0.382	0.965
									(4)x(5) = 0.156	-0.586	0.898
									(1)x(2) = 0.588	-0.018	1.194
									(1)x(3) = 0.65	0.029	1.272
									(1)x(4) = 0.032	-0.651	0.715
									(1)x(5) = 0.142	-0.542	0.825
									(2)x(3) = -0.095	-0.674	0.484
									(2)x(4) = -0.543	-1.213	0.128
									(2)x(5) = -0.487	-1.155	0.181
									(3)x(4) = -0.609	-1.294	0.075
									(3)x(5) = -0.53	-1.212	0.151
									(4)x(5) = 0.114	-0.627	0.856

To be continued

Table S3 – Mean (SD) of root mean square (RMS) and sEMG sample entropy (SaEn) values from tibialis anterior muscle for controls and neuropathy groups. F and P values for ANOVA tests. Hedges' effect sizes for comparisons between groups (conclusion).

SEMG VARIABLE	CONTROL (1)	ABSENT (2)	MILD (3)	MODERATE (4)	SEVERE (5)	P	F	η^2_p	Hedges' g effect size	95% Confidence interval (g)	
										Inferior limit	Superior limit
Tibialis anterior SaEn at 10% MVC (a.u.)	1.71 (0.17)	1.44 (0.26)	1.40 (0.31)	1.43 (0.20)	1.40 (0.34)	0.001	4.912	0.181	(1)x(2) = -1.206	-1.851	0.562
									(1)x(3) = -1.224	-1.883	0.564
									(1)x(4) = -1.532	-2.306	-0.758
									(1)x(5) = -1.224	-1.967	-0.482
									(2)x(3) = -0.140	-0.720	0.439
									(2)x(4) = -0.042	-0.701	0.618
									(2)x(5) = -0.137	-0.797	0.523
									(3)x(4) = 0.110	-0.561	0.780
									(3)x(5) = 0	-0.670	0.670
Tibialis anterior SaEn at 20% MVC (a.u.)	1.58 (0.13)	1.42 (0.23)	1.33 (0.29)	1.41 (0.21)	1.39 (0.37)	0.027	2.877	0.114	(4)x(5) = -0.108	-0.849	0.634
									(1)x(2) = -0.836	-1.455	-0.218
									(1)x(3) = -1.094	-1.744	-0.445
									(1)x(4) = -1.017	-1.741	-0.292
									(1)x(5) = -0.742	-1.447	-0.036
									(2)x(3) = -0.346	-0.928	0.237
									(2)x(4) = -0.045	-0.704	0.614
									(2)x(5) = -0.104	-0.764	0.556
									(3)x(4) = 0.305	-0.369	0.979
Tibialis anterior SaEn at 30% MVC (a.u.)	1.57 (0.11)	1.41 (0.23)	1.34 (0.21)	1.43 (0.21)	1.39 (0.32)	0.018	3.158	0.124	(3)x(5) = 0.186	-0.486	0.857
									(4)x(5) = -0.066	-0.807	0.675
									(1)x(2) = -0.862	-1.482	-0.242
									(1)x(3) = -1.353	-2.024	-0.682
									(1)x(4) = -0.884	-1.598	-0.169
									(1)x(5) = -0.815	-1.525	-0.105
									(2)x(3) = -0.317	-0.899	0.265
									(2)x(4) = 0.09	-0.57	0.794
									(2)x(5) = -0.075	-0.735	0.584
									(3)x(4) = 0.429	-0.249	1.106
									(3)x(5) = 0.194	-0.478	0.866
									(4)x(5) = -0.148	-0.89	0.594

Table S4 – Mean (SD) of root mean square (RMS) and sEMG sample entropy (SaEn) values from gastrocnemius medialis muscles for controls and neuropathy groups F and P values for ANOVA tests. Hedges' effect sizes (g) for comparisons between groups.

SEMG VARIABLE	CONTROL (1)	ABSENT (2)	MILD (3)	MODERATE (4)	SEVERE (5)	P	F	η^2_p	Hedges' g effect size	95% Confidence interval (g)	
										Inferior limit	Superior limit
Gastrocnemius medialis RMS at 10% MVC (%MVC)	3.95 (2.96)	2.72 (2.31)	4.28 (2.84)	3.47 (3.60)	2.92 (1.58)	0.273	1.309	0.056	(1)x(2) = -0.469	-1.07	0.133
									(1)x(3) = 0.114	-0.492	0.72
									(1)x(4) = -0.15	-0.866	0.567
									(1)x(5) = -0.421	-1.085	0.244
									(2)x(3) = 0.605	0.014	1.197
									(2)x(4) = 0.268	-0.427	0.964
									(2)x(5) = 0.097	-0.536	0.73
									(3)x(4) = -0.259	-0.966	0.447
Gastrocnemius medialis RMS at 20% MVC (%MVC)	4.20 (2.51)	3.44 (2.69)	4.63 (3.65)	3.32 (2.43)	3.48 (3.14)	0.581	0.719	0.031	(3)x(5) = -0.567	-1.224	0.089
									(4)x(5) = -0.209	-0.96	0.541
									(1)x(2) = -0.291	-0.888	0.305
									(1)x(3) = 0.136	-0.47	0.742
									(1)x(4) = -0.355	-1.076	0.366
									(1)x(5) = -0.257	-0.917	0.403
									(2)x(3) = 0.374	-0.21	0.957
									(2)x(4) = -0.046	-0.739	0.647
Gastrocnemius medialis RMS at 30% MVC (%MVC)	4.35 (2.93)	3.86 (4.28)	5.71 (4.23)	3.25 (2.37)	3.84 (2.23)	0.269	1.319	0.056	(2)x(5) = 0.014	-0.619	0.647
									(3)x(4) = -0.399	-1.109	0.311
									(3)x(5) = -0.334	-0.982	0.315
									(4)x(5) = 0.056	-0.693	0.805
									(1)x(2) = -0.131	-0.725	0.463
									(1)x(3) = 0.371	-0.240	0.981
									(1)x(4) = -0.402	-1.124	0.321
									(1)x(5) = -0.193	-0.852	0.466

To be continued

Table S4 – Mean (SD) of root mean square (RMS) and sEMG sample entropy (SaEn) values from gastrocnemius medialis muscles for controls and neuropathy groups F and P values for ANOVA tests. Hedges' effect sizes (g) for comparisons between groups (conclusion).

SEMG VARIABLE	CONTROL (1)	ABSENT (2)	MILD (3)	MODERATE (4)	SEVERE (5)	P	F	η^2_p	Hedges' g effect size	95% Confidence interval (g)	
										Inferior limit	Superior limit
Gastrocnemius medialis SaEn at 10% MVC (a.u.)	1.95 (0.14)	1.99 (0.09)	1.99 (0.09)	1.95 (0.14)	2.08 (0.05)	0.005	4.031	0.153	(1)x(2) = 0.347	-0.251	0.945
									(1)x(3) = 0.343	-0.267	0.953
									(1)x(4) = 0	-0.716	0.716
									(1)x(5) = 1.184	0.472	1.896
									(2)x(3) = 0	-0.579	0.579
									(2)x(4) = -0.368	-1.066	0.330
									(2)x(5) = 1.173	0.490	1.856
									(3)x(4) = -0.364	-1.073	0.344
									(3)x(5) = 1.185	0.488	1.882
									(4)x(5) = 1.318	0.493	2.142
Gastrocnemius medialis SaEn at 20% MVC (a.u.)	1.91 (0.15)	1.93 (0.19)	1.91 (0.16)	1.78 (0.14)	1.99 (0.12)	0.021	3.055	0.121	(1)x(2) = 0.116	-0.478	0.709
									(1)x(3) = 0	-0.606	0.606
									(1)x(4) = -0.888	-1.636	-0.140
									(1)x(5) = 0.582	-0.089	1.252
									(2)x(3) = -0.113	-0.692	0.466
									(2)x(4) = -0.855	-1.576	-0.135
									(2)x(5) = 0.362	-0.276	0.999
									(3)x(4) = -0.847	-1.579	-0.116
									(3)x(5) = 0.553	-0.103	1.209
									(4)x(5) = 1.630	0.768	2.492
Gastrocnemius medialis SaEn at 30% MVC (a.u.)	1.92 (0.13)	1.89 (0.20)	1.81 (0.22)	1.77 (0.15)	1.85 (0.27)	0.196	1.545	0.065	(1)x(2) = -0.175	-0.769	0.420
									(1)x(3) = -0.602	-1.221	0.018
									(1)x(4) = -1.090	-1.853	-0.326
									(1)x(5) = -0.343	-1.005	0.319
									(2)x(3) = -0.381	-0.965	0.202
									(2)x(4) = -0.648	-1.357	0.061
									(2)x(5) = -0.174	-0.808	0.46
									(3)x(4) = -0.201	-0.906	0.504
									(3)x(5) = 0.165	-0.48	0.81
									(4)x(5) = 0.352	-0.402	1.106

ANNEX 2 - SUPPLEMENTARY MATERIAL FROM PAPER 2

Table S1 – Force standard deviation (SD) and sample entropy (SaEn) values (mean±SD) for knee extension for controls and diabetic groups. F and P values for ANOVA tests. Hedge's effect sizes for comparisons between groups.

FORCE VARIABLE	CONTROL (1)	ABSENT (2)	MILD (3)	MODERATE (4)	SEVERE (5)	P	F	η ²	Hedge's g effect size	95% Confidence Interval (g)	
										Inferior limit	Superior limit
Knee extension SD at 10%MVC (N)	0.64 ± 0.37	0.90 ± 0.53	1.26 ± 0.69	1.55 ± 0.80	1.16 ± 0.48	<0.001	6.382	0.219	(1)x(2) = 0.56	-0.045	1.165
									(1)x(3) = 1.12	0.453	1.787
									(1)x(4) = 1.558	0.781	2.335
									(1)x(5) = 1.222	0.529	1.916
									(2)x(3) = 0.592	-0.014	1.199
									(2)x(4) = 1.014	0.317	1.712
									(2)x(5) = 0.51	-0.11	1.131
									(3)x(4) = 0.394	-0.296	1.083
									(3)x(5) = -0.167	-0.805	0.471
									(4)x(5) = -0.611	-1.325	0.104
Knee extension SD at 20%MVC (N)	1.14 ± 0.61	1.59 ± 0.72	1.44 ± 0.71	2.19 ± 1.12	1.77 ± 0.93	0.006	3.878	0.146	(1)x(2) = 0.669	0.06	1.279
									(1)x(3) = 0.453	-0.174	1.081
									(1)x(4) = 1.228	0.486	1.971
									(1)x(5) = 0.81	0.148	1.472
									(2)x(3) = -0.21	-0.805	0.385
									(2)x(4) = 0.678	0.001	1.354
									(2)x(5) = 0.221	-0.392	0.834
									(3)x(4) = 0.834	0.123	1.545
									(3)x(5) = 0.402	-0.241	1.045
									(4)x(5) = -0.413	-1.119	0.293
Knee extension SD at 30%MVC (N)	1.39 ± 0.82	1.84 ± 0.75	1.89 ± 0.97	2.91 ± 1.54	2.31 ± 0.99	0.001	5.392	0.192	(1)x(2) = 0.575	-0.03	1.181
									(1)x(3) = 0.557	-0.075	1.188
									(1)x(4) = 1.302	0.552	2.052
									(1)x(5) = 1.017	0.341	1.694
									(2)x(3) = 0.058	-0.535	0.652
									(2)x(4) = 0.968	0.274	1.663
									(2)x(5) = 0.545	-0.077	1.167
									(3)x(4) = 0.827	0.116	1.537
									(3)x(5) = 0.429	-0.215	1.073
									(4)x(5) = -0.477	-1.185	0.231

To be continued

Table S1 – Force standard deviation (SD) and sample entropy (SaEn) values (mean $\pm$ SD) for knee extension for controls and diabetic groups. F and P values for ANOVA tests. Hedge's effect sizes for comparisons between groups (conclusion).

FORCE VARIABLE	CONTROL (1)	ABSENT (2)	MILD (3)	MODERATE (4)	SEVERE (5)	P	F	η^2	Hedge's g effect size	95% Confidence Interval (g)	
										Inferior limit	Superior limit
Knee extension SaEn at 10%MVC (a. u.)	0.19 $\pm$ 0.11	0.18 $\pm$ 0.09	0.13 $\pm$ 0.07	0.10 $\pm$ 0.04	0.12 $\pm$ 0.05	0.001	4.890	0.177	(1)x(2) = -0.1	-0.694	0.493
									(1)x(3) = -0.651	-1.287	-0.015
									(1)x(4) = -1.017	-1.741	-0.292
									(1)x(5) = -0.805	-1.467	-0.143
									(2)x(3) = -0.613	-1.22	-0.006
									(2)x(4) = -1.055	-1.755	-0.354
									(2)x(5) = -0.793	-1.428	-0.159
									(3)x(4) = -0.503	-1.196	0.191
									(3)x(5) = -0.163	-0.801	0.475
									(4)x(5) = 0.435	-0.271	1.142
Knee extension SaEn at 20%MVC (a. u.)	0.16 $\pm$ 0.08	0.11 $\pm$ 0.04	0.12 $\pm$ 0.04	0.10 $\pm$ 0.03	0.14 $\pm$ 0.06	0.008	3.653	0.138	(1)x(2) = -0.814	-1.431	-0.197
									(1)x(3) = -0.632	-1.268	0.003
									(1)x(4) = -0.93	-1.647	-0.212
									(1)x(5) = -0.281	-0.921	0.359
									(2)x(3) = 0.25	-0.346	0.846
									(2)x(4) = -0.272	-0.934	0.39
									(2)x(5) = 0.606	-0.019	1.231
									(3)x(4) = -0.551	-1.247	0.144
									(3)x(5) = 0.396	-0.247	1.039
									(4)x(5) = 0.811	0.085	1.538
Knee extension SaEn at 30%MVC (a. u.)	0.14 $\pm$ 0.05	0.13 $\pm$ 0.06	0.14 $\pm$ 0.05	0.10 $\pm$ 0.02	0.14 $\pm$ 0.05	0.62	2.330	0.093	(1)x(2) = -0.18	-0.774	0.415
									(1)x(3) = 0	-0.62	0.62
									(1)x(4) = -0.986	-1.708	-0.264
									(1)x(5) = 0	-0.637	0.637
									(2)x(3) = 0.18	-0.415	0.774
									(2)x(4) = -0.607	-1.28	0.666
									(2)x(5) = 0.179	-0.434	0.791
									(3)x(4) = -0.986	-1.708	-0.264
									(3)x(5) = 0	-0.637	0.637
									(4)x(5) = 1.003	0.263	1.744

Table S2 – Force standard deviation (SD) and sample entropy (SaEn) values (mean $\pm$ SD) for knee flexion for controls and diabetic groups. F and P values for ANOVA tests. Hedge's effect sizes for comparisons between groups.

FORCE VARIABLE	CONTROL (1)	ABSENT (2)	MILD (3)	MODERATE (4)	SEVERE (5)	P	F	η^2_p	Hedge's g effect size	95% Confidence Interval (g)	
										Inferior limit	Superior limit
Knee flexion SD at 10%MVC (N)	0.93 $\pm$ 0.53	0.73 $\pm$ 0.31	0.67 $\pm$ 0.29	0.68 $\pm$ 0.26	0.62 $\pm$ 0.26	0.089	2.09	0.089	(1)x(2) = -0.472	-1.073	0.130
									(1)x(3) = -0.609	-1.243	0.025
									(1)x(4) = -0.555	-1.284	0.173
									(1)x(5) = -0.724	-1.391	-0.057
									(2)x(3) = -0.199	-0.794	0.396
									(2)x(4) = -0.170	-0.864	0.524
									(2)x(5) = -0.379	-1.005	0.248
									(3)x(4) = 0.036	-0.680	0.752
									(3)x(5) = -0.181	-0.829	0.467
									(4)x(5) = -0.231	-0.972	0.511
Knee flexion SD at 20%MVC (N)	1.42 $\pm$ 0.54	1.15 $\pm$ 0.66	1.31 $\pm$ 0.61	1.30 $\pm$ 0.60	1.07 $\pm$ 0.47	0.261	1.34	0.059	(1)x(2) = -0.444	-1.044	0.157
									(1)x(3) = -0.191	-0.804	0.423
									(1)x(4) = -0.213	-0.931	0.504
									(1)x(5) = -0.689	-1.344	-0.033
									(2)x(3) = 0.251	-0.337	0.839
									(2)x(4) = 0.234	-0.461	0.929
									(2)x(5) = -0.136	-0.748	0.476
									(3)x(4) = -0.016	-0.726	0.693
									(3)x(5) = -0.436	-1.073	0.201
									(4)x(5) = -0.438	-1.177	0.301
Knee flexion SD at 30%MVC (N)	1.67 $\pm$ 1.03	1.56 $\pm$ 0.85	1.64 $\pm$ 0.69	1.78 $\pm$ 0.81	1.52 $\pm$ 0.62	0.922	0.227	0.10	(1)x(2) = -0.118	-0.711	0.476
									(1)x(3) = -0.035	-0.64	0.571
									(1)x(4) = 0.115	-0.601	0.831
									(1)x(5) = -0.174	-0.812	0.464
									(2)x(3) = 0.103	-0.476	0.682
									(2)x(4) = 0.263	-0.433	0.958
									(2)x(5) = -0.052	-0.674	0.569
									(3)x(4) = 0.191	-0.514	0.896
									(3)x(5) = -0.182	-0.816	0.453
									(4)x(5) = -0.370	-1.115	0.376

To be continued

Table S2 – Force standard deviation (SD) and sample entropy (SaEn) values (mean $\pm$ SD) for knee flexion for controls and diabetic groups. F and P values for ANOVA tests. Hedge's effect sizes for comparisons between groups (conclusion).

FORCE VARIABLE	CONTROL (1)	ABSENT (2)	MILD (3)	MODERATE (4)	SEVERE (5)	P	F	η^2_p	Hedge's g effect size	95% Confidence Interval (g)	
										Inferior limit	Superior limit
Knee flexion SaEn at 10%MVC (a. u.)	0.14 $\pm$ 0.09	0.18 $\pm$ 0.09	0.20 $\pm$ 0.09	0.20 $\pm$ 0.11	0.22 $\pm$ 0.09	0.157	1.702	0.073	(1)x(2) = 0.444	-0.156	1.045
									(1)x(3) = 0.667	0.030	1.303
									(1)x(4) = 0.613	-0.118	1.345
									(1)x(5) = 0.889	0.211	1.566
									(2)x(3) = 0.222	-0.373	0.817
									(2)x(4) = 0.206	-0.488	0.901
									(2)x(5) = 0.444	-0.184	1.073
									(3)x(4) = 0	-0.716	0.716
									(3)x(5) = 0.222	-0.426	0.871
									(4)x(5) = 0.203	-0.538	0.944
Knee flexion SaEn at 20%MVC (a. u.)	0.10 $\pm$ 0.03	0.15 $\pm$ 0.09	0.10 $\pm$ 0.04	0.10 $\pm$ 0.03	0.14 $\pm$ 0.05	0.001	4.915	0.186	(1)x(2) = 0.718	0.106	1.331
									(1)x(3) = 0	-0.612	0.612
									(1)x(4) = 0	-0.716	0.716
									(1)x(5) = 0.983	0.309	1.657
									(2)x(3) = -0.702	-1.305	-0.098
									(2)x(4) = -0.658	1.368	0.051
									(2)x(5) = -0.132	-0.744	0.480
									(3)x(4) = 0	-0.709	0.709
									(3)x(5) = 0.891	0.232	1.551
									(4)x(5) = 0.925	0.158	1.692
Knee flexion SaEn at 30%MVC (a. u.)	0.10 $\pm$ 0.03	0.11 $\pm$ 0.05	0.09 $\pm$ 0.03	0.09 $\pm$ 0.02	0.12 $\pm$ 0.07	0.128	1.845	0.079	(1)x(2) = 0.237	-0.358	0.833
									(1)x(3) = -0.333	-0.943	0.276
									(1)x(4) = -0.374	-1.095	0.348
									(1)x(5) = 0.383	-0.270	1.035
									(2)x(3) = -0.480	-1.067	0.107
									(2)x(4) = -0.469	-1.170	0.233
									(2)x(5) = 0.169	-0.453	0.792
									(3)x(4) = 0	-0.703	0.703
									(3)x(5) = 0.585	-0.061	1.231
									(4)x(5) = 0.542	-0.210	1.294

Table S3 – Root mean square (RMS) and sample entropy (SaEn) values (mean±SD) for vastus lateralis for controls and diabetic groups. F and P values for ANOVA tests. Hedge's effect sizes for comparisons between groups.

SEMG VARIABLE	CONTROL (1)	ABSENT (2)	MILD (3)	MODERATE (4)	SEVERE (5)	P	F	η^2	Hedge's g effect size	95% Confidence Interval (g)	
										Inferior limit	Superior limit
Vastus lateralis RMS at 10% MVC (%MVC)	1.76 ± 0.88	1.75 ± 1.25	2.06 ± 1.29	1.60 ± 0.57	2.76 ± 1.73	0.044	2.56	0.101	(1)x(2) = -0.009	-0.603	0.584
									(1)x(3) = 0.272	-0.351	0.894
									(1)x(4) = -0.208	-0.893	0.477
									(1)x(5) = 0.741	0.083	1.399
									(2)x(3) = 0.244	-0.351	0.84
									(2)x(4) = -0.142	-0.802	0.518
									(2)x(5) = 0.686	0.057	1.314
									(3)x(4) = -0.435	-1.125	0.256
									(3)x(5) = 0.462	-0.183	1.108
Vastus lateralis RMS at 20% MVC (%MVC)	2.35 ± 1.22	2.03 ± 1.38	2.25 ± 1.41	2.36 ± 0.85	2.93 ± 1.60	0.307	1.22	0.051	(4)x(5) = 0.856	0.127	1.585
									(1)x(2) = -0.244	-0.84	0.351
									(1)x(3) = -0.076	-0.696	0.544
									(1)x(4) = 0.009	-0.674	0.692
									(1)x(5) = 0.411	-0.233	1.054
									(2)x(3) = 0.158	-0.436	0.752
									(2)x(4) = 0.271	-0.39	0.933
									(2)x(5) = 0.609	-0.016	1.234
									(3)x(4) = 0.091	-0.593	0.774
Vastus lateralis RMS at 30% MVC (%MVC)	2.42 ± 0.98	3.10 ± 1.93	2.77 ± 1.28	3.70 ± 1.59	3.29 ± 1.31	0.115	1.91	0.078	(3)x(5) = 0.453	-0.192	1.097
									(4)x(5) = 0.429	-0.277	1.136
									(1)x(2) = 0.432	-0.168	1.033
									(1)x(3) = 0.307	-0.316	0.93
									(1)x(4) = 1.013	0.289	1.737
									(1)x(5) = 0.758	0.099	1.417
									(2)x(3) = -0.198	-0.793	0.397
									(2)x(4) = 0.331	-0.333	0.994
									(2)x(5) = 0.112	-0.499	0.724
									(3)x(4) = 0.658	-0.043	1.358
									(3)x(5) = 0.402	-0.241	1.045
									(4)x(5) = -0.285	-0.987	0.417

To be continued

Table S3 – Root mean square (RMS) and sample entropy (SaEn) values (mean $\pm$ SD) for vastus lateralis for controls and diabetic groups. F and P values for ANOVA tests. Hedge's effect sizes for comparisons between groups (conclusion).

SEMG VARIABLE	CONTROL (1)	ABSENT (2)	MILD (3)	MODERATE (4)	SEVERE (5)	P	F	η^2	Hedge's g effect size	95% Confidence Interval (g)	
										Inferior limit	Superior limit
Vastus lateralis SaEn at 10% MVC (a.u.)	2.06 $\pm$ 0.05	1.97 $\pm$ 0.10	1.86 $\pm$ 0.20	1.89 $\pm$ 0.17	1.86 $\pm$ 0.26	0.001	4.94	0.178	(1)x(2) = -1.107	-1.744	-0.47
									(1)x(3) = -1.372	-2.061	-0.683
									(1)x(4) = -1.478	-2.246	-0.71
									(1)x(5) = -1.097	-1.78	-0.414
									(2)x(3) = -0.716	-1.328	-0.104
									(2)x(4) = -0.617	-1.29	0.057
									(2)x(5) = -0.592	-1.217	0.032
									(3)x(4) = 0.159	-0.525	0.843
									(3)x(5) = 0	-0.637	0.637
									(4)x(5) = -0.133	-0.832	0.566
Vastus lateralis SaEn at 20% MVC (a.u.)	1.93 $\pm$ 0.18	1.81 $\pm$ 0.20	1.81 $\pm$ 0.18	1.79 $\pm$ 0.18	1.71 $\pm$ 0.29	0.031	2.79	0.109	(1)x(2) = -0.628	-1.235	-0.02
									(1)x(3) = -0.667	-1.303	-0.03
									(1)x(4) = -0.778	-1.485	-0.07
									(1)x(5) = -0.923	-1.593	-0.253
									(2)x(3) = 0	-0.593	0.593
									(2)x(4) = -0.104	-0.763	0.556
									(2)x(5) = -0.413	-1.03	0.205
									(3)x(4) = -0.111	-0.795	0.572
									(3)x(5) = -0.42	-1.063	0.224
									(4)x(5) = -0.322	-1.025	0.381
Vastus lateralis SaEn at 30% MVC (a.u.)	1.87 $\pm$ 0.19	1.70 $\pm$ 0.22	1.70 $\pm$ 0.21	1.69 $\pm$ 0.19	1.64 $\pm$ 0.32	0.038	2.65	0.104	(1)x(2) = -0.821	-1.439	-0.204
									(1)x(3) = -0.849	-1.496	-0.202
									(1)x(4) = -0.947	-1.667	-0.228
									(1)x(5) = -0.886	-1.553	-0.219
									(2)x(3) = 0	-0.593	0.593
									(2)x(4) = -0.048	-0.707	0.612
									(2)x(5) = -0.225	-0.838	0.388
									(3)x(4) = -0.049	-0.733	0.634
									(3)x(5) = -0.224	-0.863	0.415
									(4)x(5) = -0.184	-0.884	0.516

Table S4 – Root mean square (RMS) and sample entropy (SaEn) values (mean $\pm$ SD) for biceps femoris for controls and diabetic groups. F and P values for ANOVA tests. Hedge's effect sizes for comparisons between groups.

SEMG VARIABLE	CONTROL (1)	ABSENT (2)	MILD (3)	MODERATE (4)	SEVERE (5)	P	F	η^2	Hedge's g effect size	95% Confidence Interval (g)	
										Inferior limit	Superior limit
Biceps Femoris RMS at 10% MVC (%MVC)	1.81 $\pm$ 1.49	2.49 $\pm$ 1.76	2.02 $\pm$ 1.29	2.02 $\pm$ 0.78	1.82 $\pm$ 0.86	0.495	0.854	0.038	(1)x(2) = 0.414	-0.186	1.013
									(1)x(3) = 0.151	-0.455	0.758
									(1)x(4) = 0.165	-0.552	0.881
									(1)x(5) = 0.008	-0.629	0.645
									(2)x(3) = -0.303	-0.884	0.279
									(2)x(4) = -0.310	-1.007	0.386
									(2)x(5) = -0.463	-1.082	0.156
									(3)x(4) = 0	-0.703	0.703
									(3)x(5) = -0.179	-0.803	0.445
Biceps Femoris RMS at 20% MVC (%MVC)	2.55 $\pm$ 1.96	2.56 $\pm$ 1.21	2.06 $\pm$ 1.05	2.62 $\pm$ 0.53	2.80 $\pm$ 1.58	0.398	1.027	0.046	(4)x(5) = -0.241	-0.974	0.492
									(1)x(2) = 0.006	-0.587	0.600
									(1)x(3) = -0.314	-0.930	0.302
									(1)x(4) = 0.044	-0.672	0.760
									(1)x(5) = 0.140	-0.498	0.777
									(2)x(3) = -0.439	-1.032	0.153
									(2)x(4) = 0.058	-0.635	0.751
									(2)x(5) = 0.174	-0.438	0.786
									(3)x(4) = 0.622	-0.103	1.347
Biceps Femoris RMS at 30% MVC (%MVC)	3.02 $\pm$ 2.09	3.42 $\pm$ 1.80	2.80 $\pm$ 1.36	3.38 $\pm$ 1.46	3.62 $\pm$ 2.00	0.677	0.582	0.026	(3)x(5) = 0.561	-0.081	1.202
									(4)x(5) = 0.141	-0.590	0.872
									(1)x(2) = 0.207	-0.388	0.802
									(1)x(3) = -0.126	-0.732	0.480
									(1)x(4) = 0.191	-0.526	0.908
									(1)x(5) = 0.293	-0.357	0.943
									(2)x(3) = -0.366	-0.970	0.198
									(2)x(4) = -0.024	-0.717	0.669
									(2)x(5) = 0.106	-0.516	0.728
									(3)x(4) = 0.416	-0.295	1.126
									(3)x(5) = 0.492	-0.150	1.134
									(4)x(5) = 0.133	-0.606	0.873

To be continued

Table S4 – Root mean square (RMS) and sample entropy (SaEn) values (mean $\pm$ SD) for biceps femoris for controls and diabetic groups. F and P values for ANOVA tests. Hedge's effect sizes for comparisons between groups (conclusion).

SEMG VARIABLE	CONTROL (1)	ABSENT (2)	MILD (3)	MODERATE (4)	SEVERE (5)	P	F	η^2	Hedge's g effect size	95% Confidence Interval (g)	
										Inferior limit	Superior limit
Biceps Femoris SaEn at 10% MVC (a.u.)	2.03 $\pm$ 0.04	1.97 $\pm$ 0.16	1.97 $\pm$ 0.15	1.95 $\pm$ 0.09	1.90 $\pm$ 0.17	0.024	2.977	0.122	(1)x(2) = -0.494	-1.096	0.108
									(1)x(3) = -0.547	-1.178	0.085
									(1)x(4) = -1.268	-2.048	-0.487
									(1)x(5) = -1.096	-1.789	-0.402
									(2)x(3) = 0	-0.593	0.593
									(2)x(4) = -0.142	-0.835	0.552
									(2)x(5) = -0.426	-1.055	0.202
									(3)x(4) = -0.152	-0.869	0.564
									(3)x(5) = -0.439	-1.093	0.215
									(4)x(5) = -0.377	-1.029	0.275
Biceps Femoris SaEn at 20% MVC (a.u.)	1.95 $\pm$ 0.09	1.89 $\pm$ 0.19	1.83 $\pm$ 0.28	1.75 $\pm$ 0.26	1.84 $\pm$ 0.19	0.062	2.335	0.098	(1)x(2) = -0.392	-0.991	0.207
									(1)x(3) = -0.571	-1.196	0.054
									(1)x(4) = -1.156	-1.926	-0.387
									(1)x(5) = -0.753	-1.412	-0.094
									(2)x(3) = -0.254	-0.842	0.334
									(2)x(4) = -0.651	-1.360	0.058
									(2)x(5) = -0.263	-0.877	0.351
									(3)x(4) = -0.293	-1.006	0.420
									(3)x(5) = 0.041	-0.588	0.671
									(4)x(5) = 0.409	-0.329	1.147
Biceps Femoris SaEn at 30% MVC (a.u.)	1.90 $\pm$ 0.11	1.85 $\pm$ 0.21	1.80 $\pm$ 0.29	1.72 $\pm$ 0.24	1.85 $\pm$ 0.19	0.142	1.772	0.076	(1)x(2) = -0.291	-0.887	0.306
									(1)x(3) = -0.448	-1.061	0.165
									(1)x(4) = -1.061	-1.822	-0.300
									(1)x(5) = -0.329	-0.980	0.322
									(2)x(3) = -0.199	-0.779	0.381
									(2)x(4) = -0.590	-1.297	0.116
									(2)x(5) = 0	-0.621	0.621
									(3)x(4) = -0.292	-0.999	0.415
									(3)x(5) = 0.199	-0.436	0.833
									(4)x(5) = 0.614	-0.142	1.369

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APPENDIX

Projeto de pesquisa aprovado pelo Comitê de Ética em Pesquisa da Faculdade de Medicina da Universidade de São Paulo (CEP-FMUSP)

PADRÕES DINÂMICOS DE RECRUTAMENTO MUSCULAR POR EMG DE ALTA DENSIDADE DE DIABÉTICOS EM DIFERENTES NÍVEIS DE SEVERIDADE DE NEUROPATIA CLASSIFICADOS POR UM MODELO LINGÜÍSTICO FUZZY

Dynamic muscles recruitment patterns using high-density EMG of diabetic patients in different severity degrees classified by a fuzzy system

Profa. Dra. Isabel de Camargo Neves Sacco – Coordenadora e Proponente

Laboratório de Biomecânica do Movimento e da Postura Humana - LABIMPH

Depto. Fisioterapia, Fonoaudiologia e Terapia Ocupacional, Faculdade de Medicina, Universidade de São Paulo

RESUMO

Inúmeras evidências na literatura apontam para modificações que descaracterizam o padrão clássico e estereotipado da uma marcha normal em indivíduos portadores da polineuropatia diabética, muito embora o conhecimento das causas biomecânicas deste padrão alterado de movimento ainda seja insuficiente. Além disso, estudos prévios que investigaram a biomecânica da marcha de diabéticos não têm distinguido os graus de progressão da diabetes nos grupos estudados; portanto, não é possível identificar as diferenças nos padrões de geração da marcha entre estágios precoces e avançados do desenvolvimento da doença. A presente proposta de estudo parte da premissa que a neuropatia na história clínica do diabético é um sinal da piora da doença e que, a progressão da neuropatia acaba por levar a modificações nas estratégias de controle da marcha que evoluem durante o decorrer da doença e que podem estar relacionadas aos inúmeros fatores intrínsecos biomecânicos que alteram a eficiência locomotora. Para tanto, serão interpretados os padrões eletromiográficos de diabéticos neuropatas por meio de análises no domínio do tempo, magnitude, frequência (Análise de Wavelets) e complexidade (entropia), identificando velocidade de condução, frequência de recrutamento predominante e energia, momentos distintos do apoio da marcha e em contrações isométricas de baixas intensidades. Essas análises serão feitas comparando-se os padrões encontrados através dos diferentes graus de neuropatia. Nossas teses são que (i) a lógica fuzzy irá permitir uma melhor distinção entre os diferentes estágios da doença em termos de alterações eletromiográficas, com isso possibilitará identificar alterações da dinâmica e complexidade do recrutamento muscular mesmo antes de diagnosticada a neuropatia diabética, e (ii) em estágios mais graves da neuropatia haverá uma acentuação dessas alterações.

Descritores: Eletromiografia, Neuropatias Diabéticas, Marcha, Lógica Fuzzy

ABSTRACT

Many studies have described gait alterations in individuals with diabetic neuropathy that change the classic stereotyped normal gait. However, the knowledge of biomechanical causes on this altered pattern remains insufficient. Besides that, previous studies that have investigated the gait biomechanics of diabetic patients had not distinguished the progressive degrees of the neuropathy in the studied groups. So, the identification of differences in gait alterations patterns between earlier and more advanced stages of the disease is not possible. The present study considers that the presence of

neuropathy is a sign that the diabetic disease is advancing and that the progression of the neuropathy causes modifications on control strategies of gait that evolve with the disease and might be related to intrinsic biomechanical factors that affect the locomotor efficiency. The electromyographic patterns of diabetic individuals with neuropathy will be interpreted by the means of time, magnitude and frequency domain analysis (Wavelet Analysis) and complexity analysis (entropy). The conduction velocity, the main recruitment frequencies and energy will be identified in different instants of gait stance phase and during low intensity isometric contractions. Our theses are (i) the use of Fuzzy expert model will enable a better distinction among different stages of diabetic sensorimotor polyneuropathy, and differences in muscle activity could be elucidated with this classification; (ii) in most severe disease stages the differences in muscle activity will be accentuated.

Keywords: Electromyography, Diabetic neuropathies, Gait, Fuzzy logic

1. CARACTERIZAÇÃO DO PROBLEMA

O padrão biomecânico da marcha é altamente repetitivo, cíclico e apresenta baixa variabilidade passo a passo e dia a dia (Winter, 1991), sendo por isso utilizada corriqueiramente na literatura como um modelo experimental para investigação da influência de fatores ambientais e biológicos, os quais poderiam desarmonizar as ações coordenadas dos membros e as estratégias de controle desta habilidade motora. A neuropatia diabética causa prejuízo nos sistemas somatossensorial e motor, o que modifica a quantidade e a qualidade de informação sensorial necessária para a elaboração de estratégias de geração e controle da marcha (Varraine et al., 2002). A regularidade da marcha normal permitiu que se estabelecessem critérios biomecânicos objetivos para a distinção entre padrões de movimento normais e patológicos conseqüentes da interação supostamente ineficiente de aferências e eferências já prejudicadas pela neuropatia.

A marcha do indivíduo portador de diabetes e de sua complicação crônica mais comum – a polineuropatia diabética - é alvo de estudos biomecânicos há pelo menos 20 anos (Boulton, et al., 1987) e inúmeras evidências na literatura apontam para modificações que descaracterizam o padrão clássico e estereotipado de uma marcha normal, muito embora o conhecimento das causas biomecânicas deste padrão alterado de movimento ainda é insuficiente na literatura. O conhecimento dessas causas poderia ser evidenciado pela análise dos padrões de recrutamento muscular, velocidade de condução das fibras, frequência de recrutamento, magnitude de ativação, e analisando estes parâmetros globalmente, pode-se chegar ao entendimento das estratégias de controle e coordenação muscular.

Ainda que muitas contribuições já tenham sido feitas no sentido de descrever as variáveis espaço-temporais (Courtemanche et al., 1996; Katoulis et al., 1997, Kwon et al., 2003; Menz et al., 2004), cinemáticas, de forças externas (reação do solo) (Katoulis et al., 1997; Shaw et al., 1998; Sacco & Amadio, 2000) e pressões plantares durante a marcha de diabéticos neuropatas (Lavery et al., 2003; Bacarin et al., 2009; Sacco et al., 2009; Ko et al., 2012; Sawacha et al., 2012), deve-se considerar que: (i) poucos e superficiais foram os esforços científicos para a compreensão de fenômenos internos (ações musculares) que seriam os responsáveis pela geração do padrão biomecânico externo da locomoção observado nesta população e (ii) a literatura biomecânica é inconclusiva a respeito dos efeitos da

progressão da doença.

Esses estudos prévios que investigaram a biomecânica da marcha de diabéticos não têm distinguido os graus de progressão da diabetes nos grupos estudados; portanto, não é possível identificar as diferenças nos padrões de geração da marcha entre estágios precoces e avançados do desenvolvimento da doença. Esta identificação favoreceria a intervenção terapêutica precoce nestes pacientes, o que poderia prevenir a formação de ulcerações recorrentes e consequentes amputações. Este grupo apoia a tese que estágios mais avançados da diabetes, com comprometimento real da neuropatia sensorial e motora, levariam a significativas alterações de estratégias de recrutamento muscular durante a marcha e, consequentemente da cinemática de membro inferior. Ao se identificar tais alterações biomecânicas precocemente, em estágios ainda incipientes de estabelecimento da neuropatia, poder-se-ia elaborar condutas terapêuticas e profiláticas para prevenção das ulcerações e consequentes amputações.

Sendo a diabetes a principal causa da neuropatia periférica e da amputação de membros inferiores e tratando-se de um processo insidioso e progressivo (Perkins et al., 2001) que atinge 30% dos diabéticos tipo 1 e até 40% dos diabéticos do tipo 2 (Vinik et al., 2006), tornam-se relevantes estudos que tragam novas informações sobre este quadro de progressão da doença e que permitam a intervenção precoce neste problema de saúde pública, evitando as complicações trágicas da evolução da doença.

1.1 A Diabetes Mellitus e a polineuropatia diabética

A diabetes mellitus corresponde a um grupo de doenças metabólicas caracterizadas pela hiperglicemia, causada por deficiências na secreção de insulina, da ação da insulina, ou de ambas (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003). Estima-se que cerca de 347 milhões de pessoas no mundo têm diabetes, sendo que cerca de 3,4 milhões de pessoas morreram em consequência da hiperglicemia. O número de pessoas com diabetes foi estimado em 127-182 milhões em 1980, atingindo de 314-382 milhões em 2008 (Danaei et al., 2011), e a Organização Mundial de Saúde prevê um aumento de dois terços no número de mortes por diabetes entre 2008 e 2030 (WHO, 2012). No Brasil, 5% do total de mortes são causados pela diabetes e a projeção é de que o número de mortes apresentará um aumento de 82% (WHO, 2011).

As complicações relacionadas à diabetes são muitas, uma vez que a doença pode afetar qualquer tecido do corpo humano, comprometendo principalmente o sistema cardiovascular, renal, sensorial periférico e autonômico. Entre essas complicações, estão a retinopatia, com potencial perda de visão; a nefropatia, levando a falência renal; a neuropatia autonômica causando sintomas gastrointestinais, genitourinários, sintomas cardiovasculares e disfunção sexual; e a polineuropatia diabética periférica, com risco de formação de ulcerações e subsequentes amputações (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003).

As polineuropatias diabéticas são heterogêneas, afetando diferentes partes do sistema nervoso e se apresentando com diversas manifestações clínicas. As mais comuns entre as neuropatias são as neuropatias autonômicas e a polineuropatia periférica distal simétrica. A polineuropatia diabética é definida como a presença de sintomas e/ou sinais de disfunção nervosa periférica em pessoas com diabetes após a exclusão de outras causas (Boulton et al., 2005).

Pacientes com polineuropatia periférica podem apresentar um ou mais dos seguintes sintomas: dormência, formigamento, dor ou fraqueza. Esses sintomas iniciam-se nos pés e progridem proximalmente (Callaghan et al, 2012). Indivíduos com neuropatia diabética podem apresentar sinais de anormalidades nas modalidades sensoriais primárias – dor, tato, temperatura, vibração e propriocepção; fraqueza, atrofia muscular, alterações de reflexos tendíneos, e alterações do sistema autonômico. Os sinais de perda sensorial ocorrem em uma distribuição não-dermatomal e que não correspondem à distribuição sensorial de um único nervo, e os sinais e sintomas sensoriais evoluem em uma direção centrípeta (England et al., 2005).

Os sinais motores incluem atrofia e fraqueza dos músculos intrínsecos do pé e podem estar associados a deformidades como dedos em martelo e pé cavo. Com a progressão centrípeta da neuropatia, o comprometimento motor pode evoluir para a fraqueza da extensão do hálux, seguida pela diminuição da dorsiflexão do tornozelo. Os reflexos tendíneos do tornozelo podem se encontrar diminuídos ou ausentes e os sinais de envolvimento do sistema nervoso autonômico são consistentes com o acometimento de pequenas fibras, e incluem anormalidades na sudorese e instabilidades circulatórias dos pés (England et al., 2005).

Mais de 50% dos pacientes com neuropatia diabética podem ser assintomáticos e, portanto, apresentam potencial de risco para lesões nos pés (Boulton et al., 2005). Embora as complicações no pé do diabético desenvolvam-se a partir de uma ação recíproca de vários fatores, a neuropatia parece ser o mais importante (Urbancic-Rovan, 2005). Como mais de 80% das amputações ocorrem após ulcerações ou lesões no pé, o manejo da neuropatia diabética é de suma importância (Boulton et al., 2005).

A patogênese da polineuropatia diabética é multifatorial, sendo atribuída principalmente às alterações metabólicas e da microcirculação decorrentes da exposição prolongada à hiperglicemia, associada a fatores de risco cardiovasculares (Tesfaye et al., 2010; Xu et al., 2012). A hiperglicemia persistente parece ser o fator causal mais importante no desenvolvimento da neuropatia diabética, ao levar ao acúmulo de produtos da via dos polióis (sorbitol, frutose) nos nervos, causando lesões. A diminuição da incorporação de mioinositol e a inibição da bomba NA/K/ATPase resulta em retenção de sódio e, conseqüentemente, edema da bainha de mielina, disjunção axoglial e degeneração nervosa. Além disso, a insuficiência microvascular, devido à isquemia absoluta ou relativa dos vasos do endoneuro ou do epineuro também contribuem para a lesão axonal (Gagliard, 2003; Xu et al., 2012).

Além do comprometimento sensorial, uma série de outras alterações estruturais da região distal dos membros inferiores têm sido descritas em diabéticos neuropatas. Cheing et al. (2012)

observaram um aumento do módulo de Young do tecido mole da planta dos pés de indivíduos diabéticos neuropatas, o que denota uma maior rigidez dos mesmos. D'Ambrogi et al. (2005) também descreveram um aumento da espessura tanto da fásia plantar quanto do tendão do calcâneo em indivíduos diabéticos, sendo essas alterações mais evidentes na presença da neuropatia, mas já detectável em pacientes diabéticos sem neuropatia. Além disso, foi observado nesses indivíduos uma diminuição da amplitude de movimento da articulação metatarsofalângica do hálux. Todas essas alterações provavelmente são decorrentes da glicosilação progressiva do colágeno, e prejudicam a adaptação fisiológica do pé ao solo durante a marcha. Em outro estudo, Giacomozzi et al. (2008) observaram uma diminuição da amplitude de movimento do tornozelo, relacionada a alterações estruturais da cartilagem e da cápsula articular do complexo tornozelo-pé. Os mesmos autores identificaram uma diminuição do torque isocinético do tornozelo de diabéticos neuropatas, sendo que a dorsiflexão apresentou maior comprometimento do que a flexão plantar. Essas alterações estão provavelmente relacionadas à atrofia muscular causada pela degeneração da capacidade de condução nervosa provocada pela neuropatia diabética. Gutierrez et al. (2001) avaliaram um grupo de diabéticos com neuropatia moderada, que apresentaram força normal na musculatura ao redor do tornozelo. Porém, os mesmos tinham como característica um prejuízo na função motora distal, caracterizada pela diminuição da habilidade de desenvolver força muscular rápida no tornozelo. Esses achados podem levar a instabilidade postural, e, mais uma vez, a um prejuízo da função postural e locomotora dos diabéticos neuropatas.

1.2 A progressão da neuropatia diabética e a Teoria de Conjuntos Fuzzy

O diagnóstico da polineuropatia diabética é clínico, e deve envolver o exame da sensibilidade tátil (estesiometria com monofilamento de 10g), vibratória, térmica, e reflexos do tornozelo (Boulton et al., 2005). O principal achado neurológico objetivo é a alteração da velocidade de condução nervosa, obtida através de testes eletrofisiológicos específicos (Tesfaye et al., 2010).

A gravidade da neuropatia diabética tem sido determinada através da combinação das alterações neurofisiológicas, da presença de sinais e sintomas, e da presença de outras disfunções ou prejuízo de função (Tesfaye et al., 2010). O Michigan Neuropathy Screening Instrument (MNSI-questionnaire e MNSI-form) tem se mostrado um instrumento confiável para o rastreamento e a caracterização da neuropatia, uma vez que avalia a presença de sintomas relacionados à doença, a sensibilidade vibratória, o reflexo profundo do tornozelo e a aparência de sinais nos pés – deformidades, calos, rachaduras e ulcerações prévias (Feldman et al., 1994; Moghtaderi et al., 2006). O uso da estesiometria por meio dos monofilamentos de 10g é considerada uma boa ferramenta para avaliar a perda sensorial, sendo recomendada pelo International Consensus on the Diabetic Foot (Schaper et al., 2003). Além de ter uma alta reprodutibilidade e especificidade (Sosenko et al., 1990), é um bom preditivo para riscos de ulceração e amputação (Carrington et al. 2002; Perry et al., 2002).

Os testes eletrodiagnósticos são utilizados como método objetivo de detecção de disfunção nervosa. Para a confirmação de polineuropatia diabética, o critério é de que haja uma anormalidade de qualquer atributo de condução nervosa em dois nervos diferentes, sendo que pelo menos um deles deve ser o nervo sural. Recomenda-se que os primeiros nervos a serem avaliados sejam o nervo sural e o nervo fibular (England et al., 2005). Mais recentemente, Dyck et al. (2011) observaram que os elementos mais sensíveis à neuropatia no estudo da condução nervosa, e que, portanto, apresentam diminuição de frequências nessa população, são a velocidade de condução nervosa motora dos nervos fibular, tibial e ulnar, o potencial de ação do nervo sural, e a latência da onda F dos nervos tibial e ulnar. Porém, para se definir a presença e a gravidade da polineuropatia deve-se basear-se em uma combinação de sinais, sintomas e alterações eletrofisiológicas (Tsfaye et al., 2010).

Recentemente, observou-se que há indícios claros de disfunção axonal precoce em indivíduos diabéticos sem sintomas clínicos de neuropatia, e há uma alteração progressiva nos parâmetros de excitabilidade desses nervos com a progressão da gravidade da neuropatia. Ou seja, há mudanças neurofisiológicas precoces que precedem o desenvolvimento da disfunção clínica e as mudanças estruturais nos axônios de nervos periféricos (Sung et al., 2012). Assim, mesmo a ausência de alterações em testes de condução nervosa não descartam a presença de alterações que já comprometem a função nervosa do diabético.

Na literatura não há um consenso sobre o melhor método de diagnóstico e classificação desses pacientes e cada estudo que investigou a marcha sob o ponto de vista da biomecânica utilizou uma forma diferente para definir a presença da neuropatia diabética. Existem diversos instrumentos clínicos para detectar essa complicação, como o *Michigan Neuropathy Screening Instrument* (Feldman et al, 1994; Moghtaderi et al, 2006) e *Diabetic Neuropathy Symptom Score* (Meijer et al, 2002), e alguns até apresentam uma forma de classificar a gravidade da doença, como o *Neuropathy Disability Score* (Gin et al., 2006) e o *Michigan Diabetic Neuropathy Score* (Gin et al., 2006; Lunetta et al, 1998).

Esses instrumentos fechados utilizam principalmente questionários de sintomas e testes sensoriais para avaliar os acometimentos neurológicos e definir a presença da neuropatia. As modalidades somatosensoriais mais frequentemente avaliadas são: sensibilidade tátil, em geral, testado por meio dos monofilamentos de Semmes-Weinstein de 10 g (Tan, 2010; Singh et al., 2005); sensibilidade à vibração, com uso do bioestesiômetro ou de um diapasão de 128 Hz (Perkins et al., 2001); e o reflexo calcâneo (Taksande et al., 2011). Com essa variedade de opções e diferentes formas de combinação das avaliações, é difícil haver um critério uniforme entre os estudos.

A polineuropatia sensoriomotora distal diabética geralmente tem uma instalação insidiosa, começando de forma monofásica ou flutuante ao longo do percurso da doença (Tsfaye et al., 2010), o que torna difícil a determinação do seu início. Além disso, ela se desenvolve de forma latente e subclínica em estágios muito iniciais da doença, e se agrava de forma contundente ao longo dos anos,

mostrando que a neuropatia apresenta uma progressão contínua mas não-linear (Morimoto et al., 2012). Sendo assim, os pacientes não podem ser considerados apenas como com ou sem neuropatia.

Diante desse quadro, faz-se maior a suspeita de haver uma contaminação dos grupos experimentais nos estudos prévios biomecânicos, misturando diabéticos com e sem neuropatia, e diferentes graus desse envolvimento neurológico. Além disso, mesmo ao utilizar um instrumento que avalia diversos aspectos para julgar a instalação da doença, não há critérios objetivos para interpretar essa associação de testes, e a conclusão depende da opinião subjetiva de um profissional de saúde que recebe o resultado da avaliação ou um sistema de pontuação que utiliza uma simples soma dos resultados, sem considerar o peso de cada exame. Assim sendo, a experiência do especialista da área tem um papel primordial no diagnóstico e classificação dessa doença.

A lógica fuzzy, introduzida por Lotfi Zadeh em 1965, torna-se uma ferramenta interessante para se utilizar nesse contexto, pois foi desenvolvida para lidar com problemas nos quais há incerteza na identificação dos elementos como pertencentes ou não a um determinado grupo (Zadeh, 1965).

A Teoria de Conjuntos Fuzzy é uma entidade matemática que trabalha com o conceito de “verdade parcial”, na qual as afirmações permitem uma gradação entre definições dicotômicas, formando classificações entre algo “completamente verdadeiro” e “completamente falso” (Massad, et al., 2003). Portanto, ela permite a utilização de uma faixa de contradição ou concomitância de conceitos, na qual um elemento pertence tanto a um dado conjunto quanto a outro ao mesmo tempo, com graus diferentes de pertinência. Assim, é possível combinar critérios diferentes para chegar a conclusões sobre o estado de uma doença com uma maior precisão (Licata, 2007).

A estrutura lógica da teoria de conjuntos Fuzzy permite a elaboração de variáveis linguísticas para classificar as variáveis medidas em um estudo (Massad et al., 2003). Sendo assim, a Teoria de Conjuntos Fuzzy tem como principal característica o tratamento matemático de termos linguísticos subjetivos, e trata as variáveis de estudo com base na incerteza não-probabilística (Chau et al., 2001). Esta Teoria nos permite manipular variáveis linguísticas quantificando o nível de severidade da neuropatia por meio de modelos linguísticos fuzzy, sendo, portanto, uma opção interessante para avaliar o grau de acometimento da neuropatia diabética levando-se em consideração as incertezas de identificação inerentes ao processo da doença.

Duckstein et al. (1995) desenvolveram um sistema de diagnóstico de neuropatia diabética utilizando o método Fuzzy. Foram criadas 4 classificações para os graus de neuropatia utilizando os termos linguísticos: estado normal, estado limiar, estado claro de patologia e patologia severa. Ao compararem os resultados dos diagnósticos feitos pelo modelo fuzzy com os diagnósticos médicos, encontraram 90% de concordância de resultados. Porém, foi utilizada a eletroneuromiografia como parâmetro de identificação do estado da doença. Esse método diagnóstico é um procedimento invasivo e doloroso, além de ser pouco disponível no sistema público de saúde e de necessitar de um profissional altamente especializado para a aplicação do teste. Portanto, a acessibilidade a esse recurso fica

extremamente restrita e, além disso, a detecção da doença irá ocorrer apenas quando já houver degeneração de fibras nervosas e esta alteração é irreversível.

Mais recentemente, Picon et al. (2012) desenvolveram um modelo de classificação da neuropatia diabética a partir da avaliação dos sintomas, do exame dos sinais, do nível de HbA1c e do tempo de duração da diabetes dos pacientes avaliados. O modelo sugerido permitiu classificar os pacientes de acordo com a severidade da neuropatia dos mesmos, sendo atribuído aos mesmos um escore de 0 a 10, além de poderem ser classificados em neuropatas leves, moderados ou graves após a defuzzificação. O modelo mostrou-se eficaz e tem a vantagem de ter utilizado medidas clínicas de fácil acesso como entrada, fornecendo uma ferramenta mais acessível para a detecção dos variados graus de acometimento da neuropatia periférica.

A identificação dos vários níveis de neuropatia a partir da lógica fuzzy, que leva em conta as incertezas de identificação inerentes ao processo da doença, é de fundamental importância para investigar as consequências biomecânicas da progressão da diabetes.

1.3 O padrão de ativação muscular no diabético neuropata e novas ferramentas de mensuração eletromiográfica

Destacam-se poucos estudos na literatura que usaram a eletromiografia, com o uso de eletrodos únicos bipolares, para investigar as causas das alterações observadas na fase de apoio da marcha em diabéticos neuropatas (Abboud et al., 2000; Kwon et al., 2003; Sacco & Amadio, 2003, Akashi et al., 2008; Sacco et al., 2010; Savelberg et al., 2010; Gomes et al., 2011) Isso se deve fundamentalmente porque este tipo de investigação, embora fundamental para o entendimento das causas do movimento, é extremamente complexa nesta população, considerando a dificuldade de colocação de eletrodos em músculos cujas funções já estão prejudicadas em decorrência da doença.

As alterações de estratégias de ativação muscular em diabéticos neuropatas durante a fase de apoio da marcha, embora sutis (4 - 7% de atraso de ativação na fase de apoio), restritas a alguns músculos (tibial anterior, gastrocnêmio medial e lateral e vasto lateral), e controversas entre os autores (Abboud et al., 2000; Kwon et al., 2003; Sacco e Amadio, 2003; Akashi et al., 2008; Sacco et al., 2010; Gomes et al., 2011) já são reveladoras de dificuldades em adaptar suas estratégias motoras frente aos desafios locomotores diante de seus déficits sensoriais, musculares e de movimento articular. No entanto, deve-se ter cautela ao responsabilizar alguns músculos de membro inferior por toda a complexa geração de estratégias neuromotoras necessárias para superar desafios motores das tarefas locomotoras diárias.

Os estudos eletromiográficos destacam particularmente um atraso (Akashi et al., 2008; Sacco et al., 2010) e diminuição na magnitude de ativação de gastrocnêmios (Kwon et al., 2003); uma prolongada atividade de gastrocnêmios e sóleo associada ao atraso na ativação de tibial anterior o que levaria a uma co-contração de tornozelo e pé (Kwon et al., 2003); um atraso na ativação de tibial anterior

(Abboud et al., 2000; Sacco & Amadio, 2003) e um atraso e diminuição da atividade de vasto lateral (Abboud et al., 2000; Kwon et al., 2003; Sacco & Amadio, 2003, Akashi et al., 2008). Entretanto, estas discretas alterações eletromiográficas observadas com estes eletrodos bipolares ainda são insuficientes para a compreensão das alterações cinemáticas, de força reação do solo e de pressão plantar observadas nestes sujeitos.

A interpretação do padrão eletromiográfico de músculos de diabéticos neuropatas durante a locomoção é usualmente baseada na análise estatística de variáveis discretas de envoltórios lineares ou ainda na avaliação qualitativa por inspeção visual do padrão destes envoltórios. Porém, informações a respeito das mudanças nas magnitudes, nas propriedades espectrais e na velocidade de condução ainda são ignoradas na literatura, informações essas que contribuiriam sobremaneira na investigação das estratégias de recrutamento muscular e de geração de força durante a marcha. Portanto, as alterações musculares já descritas na literatura podem ser mais reveladoras se a investigação eletromiográfica passar por uma análise multi-resolução de séries temporais no domínio do tempo, magnitude e frequência. Ou seja, ao invés de estudar a tradicional abordagem da cinemática associada à atividade elétrica muscular com eletrodos bipolares, parece ser mais promissor investigar os padrões de recrutamento, frequência de disparo e velocidade de condução das fibras musculares de membro inferior na complexa tarefa locomotora em diabéticos neuropatas por meio de eletrodos de alta densidade. Tal abordagem é realizada através de matrizes lineares ou em grade (Merletti et al., 2009).

Os eletrodos de alta densidade compreendem mais do que um único ponto de detecção ao longo das fibras musculares, diferentemente de um único ponto como os eletrodos convencionalmente utilizados em análise de movimento, particularmente em diabéticos neuropatas durante a marcha. Em cada ponto de detecção, o sinal EMG pode ser coletado de forma monopolar, ou seja, a partir de um único eletrodo em relação a um eletrodo remoto, ou ser coletado por um filtro espacial em especial, isto é, como o resultado de uma combinação linear de sinais detectados por eletrodos localizados em uma configuração geométrica específica. Os pontos de detecção do sinal EMG podem ser colocados tanto longitudinalmente às fibras musculares quanto perpendicularmente às mesmas, ou, ainda, em ambas as direções (Gazzoni et al., 2004).

Assim, com o uso destes eletrodos, aplicações clínicas são ampliadas já que se podem obter informações reveladoras sobre as propriedades de condução das unidades motoras, localização das zonas de inervação, comprimento das fibras musculares, estimação das frequências de disparo das unidades motoras e estratégias de recrutamento das fibras. Além disso, técnicas de análise EMG que se utilizam de técnicas de eletrodos que permitem a obtenção sinal com múltiplos canais de detecção apresentam melhor confiabilidade (Farina e Merletti, 2004; Farina et al., 2004), e, ao aumentarem a área de detecção do sinal EMG, permitem uma abordagem que traz uma certa compensação aos fatores geométricos que influenciam a interpretação do sinal EMG durante contrações dinâmicas, sendo a principal delas a mudança de formato do músculo durante a contração (Farina, 2006). Doenças que

resultam em alterações neurofisiológicas e consequentemente musculares, tal como a neuropatia diabética, se beneficiariam de tal abordagem de investigação que proporcionaria dados adicionais para a melhor compreensão dos mecanismos centrais e periféricos de controle do movimento nestas doenças (Merletti et al., 2003, Farina e Merletti, 2004).

1.4 Novas ferramentas de análise do sinal biológico: complexidade e variabilidade

A dinâmica dos vários processos fisiológicos é inerentemente complexa. Os sistemas biológicos são regulados por mecanismos que operam através de escalas múltiplas no tempo e no espaço e, portanto, sua complexidade também é multiescalar e hierárquica (Costa et al., 2005). Entende-se por complexidade a presença de flutuações não randômicas em escalas temporais múltiplas na dinâmica aparentemente irregular dos outputs fisiológicos (Manor et al., 2010), tal como um sinal eletromiográfico.

A complexidade fisiológica está fundamentalmente relacionada à capacidade adaptativa do organismo, que requer funcionalidade integrativa e multiescalar. As medidas de complexidade fisiológica têm sido relacionadas à funcionalidade do sistema ao serem definidas como a capacidade de se gerar repostas adaptativas aos elementos estressores, de se adaptar e funcionar em um ambiente que está em constante mudança (Peng et al., 2009; Manor et al., 2010). A locomoção em ambientes com obstáculos físicos ou em condições neuromusculares desfavoráveis devem produzir respostas musculares e, consequentemente mecânicas, tais que o sistema adapte-se às demandas da tarefa e ambiente, muito embora tal mecanismo pode não ter êxito completo. Estados patológicos, como a neuropatia diabética, assim como o envelhecimento, aparentemente degradam essa complexidade biológica reduzindo, assim, a capacidade de adaptação do sistema (Peng et al., 2009).

A noção de complexidade apresenta parentesco próximo à de conteúdo de informação, na Teoria da Informação. O conteúdo de informação é quantificado pela entropia. Quanto maior for a entropia de informação de uma série temporal, mais complexa ela é (Duarte e Stenard, 2008). Os algoritmos baseados na entropia quantificam a regularidade de uma série temporal. A entropia aumenta com o grau de desordem e é máxima para sistemas completamente randômicos. A entropia de uma variável randômica discreta é uma medida de sua incerteza média (Costa et al., 2002).

Estados patológicos e o envelhecimento podem ser definidos por um esgotamento sustentado de correlações de grande espectro, e perda de informação. Assim, a dinâmica patológica está associada tanto com um aumento da regularidade / diminuição da variabilidade, quanto com o aumento da variabilidade devido à perda das propriedades de correlação. Ambas as situações são caracterizadas por uma diminuição da entropia (Costa et al., 2002).

Uma série de variáveis foram desenvolvidas a fim de analisar a entropia de sistemas biológicos. A *sample-entropy* fornece uma medida de uma “estrutura ordenada” em uma série temporal ao testar se há quaisquer padrões repetidos de comprimentos variados, incluindo aqueles que podem não se

repetir a intervalos regulares (Duarte e Stenard, 2008). Corresponde ao logaritmo negativo natural para a probabilidade condicional de que duas sequências similares para n pontos se mantêm similares ao próximo ponto, onde “*self-matches*” não são incluídos no cálculo da probabilidade. Quanto menor a *sample-entropy*, maior a similaridade própria na série temporal, e, portanto, menor a complexidade (Richman e Moorman, 2000). A *multiple-scale-entropy* (MSE) mede o conteúdo da informação de uma série temporal complexa em escalas temporais múltiplas (Duarte e Stenard, 2008). Ela se baseia na observação de que o output de sistemas complexos está longe do extremo da regularidade perfeita, assim como da randomização completa. Ao invés disso, esses sistemas geralmente revelam estruturas com correlações de grande amplitude em escalas temporais e espaciais múltiplas (Costa et al., 2005).

Assim, examinar a complexidade da atividade muscular dos diabéticos neuropatas ao longo do desenvolvimento da neuropatia pode trazer uma nova visão do efeito da patologia no controle motor desses indivíduos. Além disso, essa nova variável poderá ser analisada e comparada entre os diversos grupos neuropatas, ou seja, ao obtermos uma nova medida do padrão EMG para os indivíduos com os variados graus de neuropatia diabética, será possível tentar identificar se os mesmos apresentam um padrão de atividade muscular de acordo com a neuropatia diabética; nesse caso, um nível de complexidade de atividade muscular, que mudaria de acordo com o grau da neuropatia. A Análise de Cluster, por exemplo, é uma técnica desenvolvida para identificar agrupamentos naturais que podem existir em uma população de interesse. Assim, ela permite alocar indivíduos em um grupo que contém outros indivíduos com características semelhantes, a partir da análise das variáveis descritivas para cada indivíduo, que permite determinar as similaridades e as diferenças entre eles. O método permite, portanto, o descobrimento de informações ou padrões úteis, mas não óbvios, em grandes conjuntos de dados, e possibilitaria a investigação da existência de padrões EMG entre os diferentes níveis de neuropatia – nesse caso, estudar-se-ia se há um padrão de complexidade característico para o nível de neuropatia diabética.

2. OBJETIVOS E TESTES DO PROJETO

A presente proposta de estudo parte da premissa que a neuropatia na história clínica do diabético é um sinal da piora da doença e que, a progressão da neuropatia acaba por levar a modificações nas estratégias de controle da marcha que evoluem durante o decorrer da doença e que podem estar relacionadas aos inúmeros fatores intrínsecos biomecânicos que alteram a eficiência locomotora. A análise dos padrões de recrutamento muscular, velocidade de condução das fibras, frequência de recrutamento, magnitude de ativação e entropia pode levar a um melhor entendimento das estratégias de controle e coordenação muscular. Desta forma, tem-se como metas investigar as alterações nos padrões dinâmicos de atividade muscular durante a marcha e em condições estáticas em contrações isométricas de baixas intensidades (0 a 10% 20 e 30% da máxima), por meio de eletrodos de

matriz em grade, que podem ocorrer nos estágios mais iniciais da neuropatia diabética, em uma fase ainda subclínica, e acompanhar essas alterações com a progressão da severidade da neuropatia.

2.1 Objetivo geral

Investigar os padrões dinâmicos e estáticos de recrutamento muscular durante a marcha e em contrações isométricas de baixa intensidade, e a complexidade da atividade muscular de membro inferior, expressa através da entropia, em diabéticos com diferentes estágios da polineuropatia periférica, classificados por meio de um sistema baseado na lógica fuzzy.

2.2 Objetivos específicos

- Avaliar e comparar os padrões temporais de recrutamento muscular durante a marcha de vasto lateral, tibial anterior, gastrocnêmio lateral, bíceps femoral em indivíduos diabéticos em diferentes níveis de severidade da neuropatia;
- Avaliar e comparar os padrões de frequência de recrutamento das fibras musculares durante a marcha de vasto lateral, tibial anterior, gastrocnêmio lateral, bíceps femoral em indivíduos diabéticos em diferentes níveis de severidade da neuropatia;
- Avaliar e comparar as velocidades de condução das fibras musculares durante fases específicas da marcha e durante as contrações isométricas de 0 a 10% em rampa, 20% e 30% da contração isométrica máxima de vasto lateral, tibial anterior, gastrocnêmio lateral, bíceps femoral em indivíduos diabéticos em diferentes níveis de severidade da neuropatia;
- Avaliar e comparar a entropia nas contrações isométricas de baixas intensidades de vasto lateral, tibial anterior, gastrocnêmio lateral, bíceps femoral em indivíduos diabéticos em diferentes níveis de severidade da neuropatia.

2.3 Teses

Nossas teses são que (i) a lógica fuzzy irá permitir uma melhor distinção entre os diferentes estágios da doença em termos de alterações eletromiográficas, com isso possibilitará identificar alterações da dinâmica e complexidade do recrutamento muscular mesmo antes de diagnosticada a neuropatia diabética, e (ii) em estágios mais graves da neuropatia haverá uma acentuação dessas alterações.

3. METODOLOGIA E ESTRATÉGIAS DE AÇÃO – MATERIAIS E MÉTODOS

3.1. Desenho experimental

Estudo laboratorial transversal (cinco grupos) onde o fator doença (variável independente) será apresentado em cinco níveis em: i) não doente, ii) diabético, iii) diabético com neuropatia leve, iv) diabético com neuropatia moderada, v) diabético com neuropatia grave. Estes grupos serão relacionados com o grupo de variáveis dependentes providas da EMG superfície de alta densidade (séries temporais). As hipóteses a serem testadas para os grupos estudados serão:

$$H_0: \lambda_1 = \lambda_2 = \lambda_3 = \lambda_4 = \lambda_5 = 0 \text{ e}$$

$$H_A: \text{no mínimo um } \lambda_i \neq 0,$$

onde λ_i , $i = 1, 2, 3, 4, 5$ representando os efeitos do fator doença.

3.2 Casuística

Os grupos do estudo serão formados por 75 voluntários adultos, não idosos, de ambos os sexos, distribuídos em: 15 diabéticos sem diagnóstico ou sintomas de neuropatia periférica (GD), 15 diabéticos com sintomas leves de neuropatia periférica (GDNL), 15 diabéticos com sintomas moderados de neuropatia diabética (GDNM), 15 diabéticos com sintomas graves de neuropatia diabética (GDNG) e 15 indivíduos não diabéticos (GC) com idade, distribuição de sexo e massa equivalentes aos outros grupos. Os indivíduos diabéticos serão provenientes da ANAD – Associação Nacional de Assistência ao Diabético - uma instituição filantrópica com mais de 25 anos que conta atualmente com 15 mil associados, cujo objetivo é atuar junto aos portadores de diabetes. Os 4 grupos de diabéticos serão classificados pelo modelo fuzzy desenvolvido.

Os critérios de exclusão adotados para ambos os grupos serão: indivíduos com idade superior a 65 anos, presença de amputação de qualquer parte do pé ou incapacidade de deambulação independente. Além disso, serão excluídos também sujeitos com artropatia de Charcot, presença de úlceras plantares ativas no momento dos testes ou portadores de claudicação por qualquer razão, história de vestibulopatia, doença neurológica de origem central ou periférica que não seja causada pela diabetes mellitus (poliomielite, neuropatia alcoólica, retinopatia, nefropatia). Indivíduos com retinopatia diabética também serão excluídos, uma vez que o déficit de visão pode alterar os padrões da marcha.

Todos os voluntários que participarão pesquisa irão assinar um termo de consentimento livre e esclarecido, elaborado conforme resolução 196/96 do Conselho Nacional de Saúde, ficando uma cópia com o voluntário e outra com o pesquisador. Este termo obedece às características metodológicas éticas orientadoras deste projeto.

3.3 Protocolo de avaliação

O protocolo será desenvolvido no Laboratório de Biomecânica do Movimento e Postura Humana do Departamento de Fisioterapia, Fonoaudiologia e Terapia Ocupacional da Faculdade de Medicina da Universidade de São Paulo, e será constituído de 3 etapas: (1) avaliação inicial para investigação de aspectos relacionados à diabetes e à neuropatia, (2) classificação dos sujeitos diabéticos

entre os grupos GD, GDNL, GDNM e GDNG através de um modelo linguístico fuzzy e (3) avaliação eletromiográfica dinâmica (marcha em cadência auto-selecionada) e estática (Figura 1).

Figura 1 – Fluxograma do protocolo experimental

3.4 Avaliação inicial

Além do diagnóstico clínico fornecido pela equipe médica dos ambulatórios envolvidos, todos os indivíduos serão entrevistados e examinados clinicamente para investigação de aspectos relacionados à diabetes e a neuropatia, por meio de um questionário, o MNSI *-questionnaire* e exame físico dos pés, pelo MNSI- *form* (Feldman *et al.*, 1994; MNSI, 2008), com confiabilidade e reprodutibilidade confirmada por estudos de Lunetta *et al.* (1998). Nesta etapa também serão coletados, por meio de uma entrevista, os dados pessoais e os referentes à doença: se diabético ou não, tipo de diabetes, tempo de diagnóstico da doença, história prévia de ulceração, medicamentos em uso, presença de complicações (retinopatia ou nefropatia). Além da entrevista far-se-á a mensuração da percepção à vibração no hálux (Boulton *et al.*, 1986; Zimny *et al.*, 2001; van Schie *et al.*, 2004) e do reflexo aquileu e da hemoglobina glicada por meio de um monitor (A1CNow, Bayer, Leverkusen, Alemanha). Esta avaliação inicial servirá de base para verificar os critérios de inclusão e exclusão dos grupos e para caracterizar melhor os sujeitos participantes do estudo.

3.5 Modelo fuzzy e o processo de classificação

Para a classificação da gravidade da neuropatia diabética, será utilizado um sistema baseado em regras fuzzy, aprimorado a partir do modelo de Picon *et al.* (2012). O processo desse sistema é composto por quatro fases:

- 1) Módulo de fuzzificação, que traduz valores de medição clássica (entradas crisp) das variáveis de entrada (inputs) em conjuntos fuzzy, representados por variáveis linguísticas;
- 2) Módulo de base de regras, no qual as variáveis de entrada e suas classificações linguísticas são modeladas por funções de pertinência, por meio de regras linguísticas do tipo Se-Então (proposições fuzzy);
- 3) Módulo de inferência fuzzy, cujo papel é traduzir matematicamente as proposições fuzzy e definir quais regras serão utilizadas para estabelecer a relação fuzzy entre as variáveis de entrada, e
- 4) Módulo de defuzzificação, em que a modelagem matemática realizada no módulo de inferência pela relação fuzzy é transformado em um número real de valor crisp novamente (output) (Barros e Bassanezi, 2006).

O modelo fuzzy foi dividido em duas partes, cada uma elaborada com base na opinião de uma equipe com quatro especialistas em neuropatia diabética. Esses especialistas apresentavam no mínimo 10 anos de experiência em avaliação e classificação deste tipo de paciente, e construíram cada parte do sistema de forma consensual. A primeira fase gera uma saída defuzzificada representativa da evidência de neuropatia diabética, utilizando como variáveis de entrada a sensibilidade vibratória, tátil e os sintomas típicos da neuropatia diabética. O valor defuzzificado (output) de evidência de neuropatia serve de input, junto com o tempo de diabetes mellitus e o resultado da inspeção visual dos pés, para a

segunda parte do modelo, cuja função é realizar um refinamento da primeira parte para gerar a saída final, que gera o grau de neuropatia diabética (Figura 2).

Figura 2 – Processo de modelagem do grau de neuropatia diabética no qual: (I) a primeira fase tem como variáveis de entrada a sensibilidade vibratória, tátil e sintomas da neuropatia, e gera a variável de saída de evidência de neuropatia; e (II) a segunda fase utiliza como entrada a evidência de neuropatia gerada no passo anterior, o tempo de diabetes e o grau de alteração dos pés, resultando no grau de neuropatia.

Os resultados de cada teste da avaliação clínica são transformados em escalas numéricas para serem utilizados como variáveis de entrada no sistema e essa definição também foi realizada pelos especialistas. Para a sensibilidade vibratória, é utilizada uma escala de 6,0 (seis) pontos, na qual se soma 3,0 (três) pontos para uma sensibilidade “ausente”, 1,0 (um) para “diminuída” e nenhum para “presente”. Na sensibilidade tátil, considera-se uma escala de 4,0 (quatro) pontos e cada área onde o filamento não é percebido soma 0,5 (meio) ponto. Quanto à avaliação de sintomas, é verificada a

presença de sensações de dormência, queimação, agulhadas e formigamento, cada um somando 1,0 (um) ponto; dor ou incômodo com o contato do lençol com a pele dos pés e diminuição ou ausência de sensação dos pés durante o andar, somando 2,0 (dois) pontos cada; e piora dos sintomas à noite e/ou com repouso, somando 2,0 (dois) pontos quando ocorrem isoladamente e 3,0 (três) quando ambos estão presentes.

Os inputs da primeira parte do modelo são fuzzificados pelas seguintes variáveis linguísticas (Figura 3):

(a.1) *Avaliação da sensibilidade vibratória*: (1) pouco alterada, (2) alterada ou (3) muito alterada;

(b.1) *Avaliação da sensibilidade tátil*: (1) pouco alterada, (2) alterada ou (3) muito alterada;

(c.1) *Avaliação dos sintomas*: (1) muito fraco, (2) fraco, (3) moderado ou (4) forte.

A evidência de neuropatia, gerada pela combinação dos parâmetros da primeira parte do modelo, é representada por um número real no intervalo [0, 10], fruto da defuzzificação da primeira parte do sistema. Para a medida das alterações observadas na inspeção dos pés, utiliza-se uma escala de 9,0 (nove) pontos, com a presença de deformidades nos dedos pontuando 4,0 (quatro) no caso de dedos em garra ou em martelo e 1,0 (um) para hálux valgo; 2,0 (dois) pontos para pés cavos e 1,0 (um) ponto para pés planos, e a presença de calos e rachaduras somando 1,0 (um) ponto cada. O tempo de diabetes é inserido no sistema em forma de anos desde a confirmação do diagnóstico da doença.

O output da primeira parte do modelo e os inputs da segunda, seguem as próximas variáveis linguísticas na fuzzificação (Figura 4):

(a.2) *Evidência de neuropatia*: (1) muito baixa, (2) baixa, (3) moderada, (4) alta ou (5) muito alta;

(b.2) *Tempo de diabetes*: (1) curto, (2) moderado e (3) longo;

(c.2) *Alteração dos pés*: (1) muito leve, (2) leve, (3) moderada, (4) grave ou (5) muito grave.

O output ao final dessas duas partes indica o grau de neuropatia por um número real no intervalo [0,10] novamente, e utiliza-se os conjuntos fuzzy representados para a defuzzificação, que também foram definidos por consenso dos especialistas, utilizando estes termos linguísticos: neuropatia ausente, leve, moderada, elevada e grave. As saídas geradas em cada parte do sistema foram definidas por meio de uma análise combinatória dos conjuntos de entrada, que definia regras linguísticas, cuja função era atrelar a combinação dos inputs a um conjunto fuzzy do output. Assim, foram elaboradas 36 regras na primeira parte do modelo e 75 na segunda, utilizando a forma dos seguintes exemplos:

Exemplo da primeira parte do modelo:

Regra para determinação da evidência de neuropatia

“SE a sensibilidade à vibração é *pouco alterada* E a sensibilidade tátil é *pouco alterada* E os sintomas são *moderados*, ENTÃO a evidência de neuropatia é *baixa*.”

Exemplo da segunda parte do modelo:

Regra para determinação do grau de neuropatia

“SE a evidência de neuropatia é *baixa* E o tempo de diabetes é *moderado* E a alteração dos pés é *leve* ENTÃO o grau de neuropatia é *leve*.”

Por meio dessas regras, determinados conjuntos de saída são ativados, delimitando uma área em cada um desses conjuntos, definida pela largura da sua base e a altura, cujo valor é o grau de pertinência a esse conjunto (método de inferência de Mamdani). As áreas desses conjuntos são então sobrepostas e a projeção ortogonal do centro da figura resultante sobre o eixo de pontuação da saída define o valor defuzzificado do output (método de defuzzificação por centro de área).

Para possibilitar a alocação dos indivíduos diabéticos nos grupos experimentais, a variável de saída é categorizada sob os seguintes termos linguísticos dos conjuntos fuzzy de saída por meio desta estratificação, sendo o valor de saída representado por x : (i) neuropatia ausente para $x \leq 2,5$; (ii) neuropatia leve para $2,5 < x \leq 5,0$; (iii) neuropatia moderada para $4,5 < x \leq 8,5$, e (iv) neuropatia grave para $8,5 < x \leq 10,0$.

No caso de presença de história de ulceração plantar, o indivíduo não passa pela avaliação do sistema fuzzy e é classificado automaticamente como um caso de neuropatia grave, já que esta é uma consequência que tem alta relação com estados avançados da doença.

Figura 3 – Conjuntos fuzzy das variáveis de entrada da primeira parte do modelo: sensibilidade vibratória, sensibilidade tátil e sintomas da neuropatia.

Figura 4 – Conjuntos fuzzy das variáveis de entrada da segunda parte do modelo: evidência de neuropatia, tempo de diabetes e grau de alteração dos pés.

3.6 Avaliação eletromiográfica estática e dinâmica

A atividade eletromiográfica dos músculos vasto lateral (VL), gastrocnêmio lateral (GL) e tibial anterior (TA) e bíceps femoral (BF) será mensurada do membro inferior escolhido aleatoriamente, para evitar que a dominância interfira nos resultados. Estes músculos foram selecionados para este estudo devido ao papel fundamental que desempenham na marcha. A atividade EMG dos músculos VL, GL e TA é freqüentemente reportada na literatura, em consequência da menor impedância da gordura corporal e nos permite realizar comparações com o que vem sendo discutido na literatura. Já o BF, que também possui papel fundamental na progressão da marcha, como antagonista do quadríceps, não é reportado na literatura com esta população já que apresenta maior influência da gordura corporal, maior nessas regiões em diabéticos neuropatas. Porém optou-se por analisar no presente estudo devido sua importância para compreensão de estratégias de coordenação muscular intra-membro. Músculos do quadril (glúteo máximo e tensor da fáscia lata), que também possuem papel fundamental na progressão da marcha, cuja investigação seria interessante, apresentam maior influência da gordura corporal, maior nessas regiões em diabéticos neuropatas, o que poderia comprometer os resultados.

Os sinais de EMG de superfície serão detectados utilizando uma matriz linear de 64 eletrodos com 8 mm de distância intereletrodos na configuração de memória SD (ELSCH064NM2). A matriz será conectada a um amplificador EMG multi-canais (EMG amplificador-256, com CMRR > 100 dB, ganho variável de 100 a 50.000, e o nível de ruído equivalente de entrada inferior a 1 μ VRMS; OT Bioelettronica, Torino, Itália) (Figura 5). Os sinais EMG serão amplificados, filtrados com passa-banda (3 dB largura de banda, 10-500 Hz), amostrados a 2048 Hz (conversor A/D 12-bits) e armazenados em um computador.

Figura 5 - Amplificador do eletromiógrafo [à esquerda], matriz linear com 64 eletrodos [à direita].

Como preparação para a colocação das matrizes (figura 5) será realizada a tricotomia e a abrasão da pele com gaze embebida em álcool. Cabe ressaltar aqui que a abrasão da pele será realizada cautelosamente, principalmente nos diabéticos neuropatas, a fim de não causar nenhuma lesão nestes sujeitos.

As matrizes serão fixadas à pele através de uma espuma adesiva específica (KITAD004, OT Bioelettronica, Torino, Itália) e os espaços criados por esta espuma entre a pele e a interface, que são cavidades retangulares serão preenchidos por 20 a 30 μ l de gel condutor (CC1, OT Bioelettronica, Torino, Itália), garantindo um contato adequado com a pele e atuando como atenuador no caso de

movimentação do eletrodo. O local de fixação será determinado de acordo com Farina et al. (2004). Localizar-se-á a zona de inervação dos músculos a serem estudados, e matriz será colocada entre a zona de inervação detectada mais distalmente e o tendão distal. A orientação da matriz será determinada com base na inspeção visual, de forma que escolher-se-á o ângulo de inclinação que levar aos potenciais de ação mais similares viajando ao longo das colunas, aumentando a confiabilidade da estimativa da velocidade de condução das fibras musculares (Farina, 2004). O eletrodo de referência será fixado em volta do punho.

Com o indivíduo sentado no ergômetro de joelho instrumentado com célula de carga, ele realizará a contração voluntária isométrica máxima (CVIM) contra resistência do ergômetro para flexão e para extensão e a célula de carga registrará a força exercida pelo grupamento muscular correspondente. Repetirá o mesmo procedimento para avaliação da CVIM no ergômetro em formato de pedal para flexão e extensão de tornozelo. Após a aquisição de duas tentativas para cada músculo da CVIM, os indivíduos repetirão os testes contra resistência dos ergômetros correspondentes (joelho e tornozelo) e exercerão uma contração isométrica em rampa de 0 a 10% da CVIM; posteriormente em 20% da CVIM e em 30% da CVIM. Para cada uma das condições (10, 20 e 30%) serão adquiridas três tentativas de 3 segundos cada. Durante a avaliação das contrações isométricas, a aquisição da EMG será sincronizada por meio de um conversor A/D de 12 bits de resolução (placa de aquisição OT) com o sinal da célula de carga de cada ergômetro (joelho e tornozelo). Destes sinais das contrações isométricas serão calculados: (a) velocidade de condução e (b) entropia.

Previamente à avaliação da marcha, os indivíduos serão instruídos a andar livremente pela passarela de 10 m do laboratório em cadência auto-selecionada, para adaptação ao ambiente e reprodução da marcha cotidiana. A velocidade será monitorada e mensurada por meio de dois sensores fotoelétricos (CEFISE, Modelo Speed Test Fit, Nova Odessa, Brasil) colocados equidistantes na pista de 10 m. Serão coletados três tentativas (apoios da marcha), quantidade esta recomendada por Arsenaut et al. (1986), devido à baixa variabilidade intra-indivíduo de passada a passada. Destes sinais musculares adquiridos durante a marcha serão calculados: (a) velocidade de condução em fases específicas da marcha, (b) espectro de frequência e (c) energia do sinal.

Durante a avaliação da marcha, a aquisição da EMG será sincronizada por meio de um conversor A/D de 12 bits de resolução (placa de aquisição OT) com o sinal de dois eletrogoniômetros que registrarão a variação angular sagital de tornozelo e joelho (modelo SG110/A e SG 150, Biometrics, Gwent, Inglaterra) e com a componente vertical da força reação do solo obtida por uma plataforma de força (AMTI, OR6-7-2000, EUA) (Figura 6). O sinal EMG, dos eletrogoniômetros e da plataforma de força serão amostrados a 2048 Hz (conversor A/D 12-bits). O instante do contato do calcanhar com a plataforma (início da componente vertical da força reação do solo) será utilizado como referência para o início do apoio da marcha, e a retirada dos dedos e, portanto o instante final da força reação do solo vertical, será utilizado como referência para determinar o final da fase de apoio na marcha. Esta

definição da fase de apoio da marcha será utilizada para a análise de Wavelets do sinal eletromiográfico.

Figura 6 – Passarela para coleta de dados durante a marcha (a) e plataforma de força modelo OR61000, AMTI (b).

O sinal da variação angular de tornozelo e joelho será usado para definir os ângulos e fases da marcha em que será calculada a velocidade de condução muscular: (a) zeros graus extensão de joelho e 90° flexão de tornozelo no contato inicial da marcha, (b) zeros graus extensão de joelho e 90° flexão de tornozelo no médio apoio, (c) 50° graus extensão de joelho e 20° extensão de tornozelo na fase de propulsão.

Os eletrogoniômetros são constituídos por dois blocos terminais ligados por uma mola instrumentada com uma célula de carga, posicionados no joelho e tornozelo. Conforme a orientação do fabricante (Biometrics, 2002), na articulação do joelho os blocos serão fixados seguindo a diáfise do fêmur e da fíbula, já para o tornozelo, os blocos serão fixados no bordo lateral da perna, em direção a cabeça da fíbula e no bordo lateral do pé, logo à frente ao maléolo lateral. A célula de carga será mantida em tensão e o seu centro deve coincidir aproximadamente com o eixo das articulações. Com os sensores já posicionados no sujeito, o equipamento é calibrado. Para definição do zero dos goniômetros, o voluntário será orientado a manter postura ortostática, peso corporal distribuído igualmente entre os dois pés e equilíbrio estacionário. Movimentos que geram diminuição do ângulo articular são definidos como flexão (valores positivos) e movimentos que geram aumento do ângulo articular, como extensão (valores negativos) (Winter, 1991).

3.7 Análise matemática dos dados eletromiográficos

As variáveis EMG serão calculadas por meio de rotinas personalizadas criadas em ambiente

Matlab v. 7.6/R2008a (MathWorks, Inc.).

As variáveis da EMG calculadas das contrações isométricas serão: (a) a velocidade de condução de cada músculo em cada condição de contração (10, 20 e 30% da CVIM), e (b) entropia do sinal. As variáveis da EMG calculadas da fase de apoio da marcha serão: (a) energia em cada faixa de frequência definida pela análise de wavelets, (b) a frequência central na maior energia durante o apoio, (c) a velocidade de condução nos três momentos e ângulos de tornozelo e joelho, (d) a entropia.

Análise de Wavelets

O sinal EMG é um sinal não-estacionário, ou seja, as frequências do sinal mudam ao longo do tempo. A transformação de wavelets fornece uma análise de séries temporais no domínio do tempo e da frequência, que acaba por refletir os padrões e estratégias de recrutamento muscular e geração de força ao longo de todo o ciclo da habilidade motora a ser analisada.

A transformação discreta de wavelet (DWT) opera sob um sinal que apresenta valores em instantes discretos, e não contínuo, ao longo do tempo. O comprimento de um sinal discreto corresponde ao número de instantes ao longo do qual o sinal foi medido.

Havendo-se um sinal $x(n)$ de comprimento N e frequência máxima f_{max} , a transformação se inicia decompondo-se o sinal $x(n)$ em dois subsinais, chamados de sinal de tendência (“trend signal”) e sinal de flutuação (“fluctuation signal”), cada um deles com comprimento $N/2$. O sinal flutuante d_1 contém a metade superior das frequências do sinal original, ele é obtido aplicando-se ao sinal original um filtro passa-alta ou um filtro passa-banda $[f_{max}/2, f_{max}]$. De forma similar, o sinal de tendência a_1 é obtido aplicando-se ao sinal original um filtro passa-baixa $[0, f_{max}/2]$. Esses dois subsinais constituem o primeiro nível de decomposição. Os subsinais correspondem a uma subamostra de fator 2, o que significa que qualquer outra subamostra é descartada. Isso é justificado pelo Teorema de Nyquist; uma vez que a largura da banda de cada subsinal é metade à do sinal original, os subsinais contêm, na verdade, mais amostras do que o necessário para se capturar os componentes de frequência que constituem os subsinais. A decomposição é, então repetida apenas para o primeiro sinal de tendência a_1 para produzir um segundo sinal de tendência a_2 e um segundo sinal de flutuação d_2 , e, novamente, a largura da banda e o comprimento do sinal são divididos pela metade. Esse processo é repetido nos sinais de tendência subsequentes até que um subsinal de comprimento 1 seja obtido. Para um sinal de comprimento N , haverá $K=\log N/\log 2$ níveis de decomposição. Finalmente, a transformação discreta de wavelet é obtida pela concatenação do último sinal de tendência com todos os sinais de flutuação (Chau, 2001b).

$$DWT[x(n)] = (a_K | d_K | d_{K-1} | \dots | d_2 | d_1)$$

A DWT permite, assim, a análise de eventos específicos em diferentes bandas de frequência dentro do sinal EMG, enquanto a resolução temporal é mantida. A transformação decompõe o sinal em uma combinação linear de funções wavelet que permitem estimar a quantidade de energia em cada banda de frequência, e ambos os parâmetros serão determinados.

Velocidade de condução

Para o cálculo da velocidade de condução, um detector estatístico de duplo limite (Xu e Adler, 2004) será utilizado para inferir os intervalos de ativação muscular dos canais e será definido como período de atividade muscular quando todos os sinais de uma mesma matriz acusar atividade mioelétrica (Farina, 2004).

Canais duplo-diferenciais serão computados através da subtração do sinal diferencial simples obtido e será computada a velocidade de condução através do algoritmo de máxima semelhança (*maximum likelihood*), onde os sinais serão empareados em interações sucessivas até ser encontrado o pareamento que resulte no mínimo erro quadrado médio (*mean square error* - MSE) entre os sinais, sendo que esta mudança no tempo será utilizada no cálculo da velocidade de condução das fibras musculares (*muscle fiber conduction velocity* - CV) (Farina, 2004). Este cálculo será feito em janelas gaussianas sucessivas de 30ms de desvio padrão a cada 50ms do intervalo de tempo definido como atividade muscular para cada músculo. O máximo da função de correlação cruzada entre pares de sinais será usado para inferir a similaridade do sinal, que se reflete na qualidade da medida de CV.

Cálculo da entropia

A entropia será calculada para a atividade EMG dos músculos estudados utilizando-se a multiscale entropy (MSE) de acordo com Costa et al. (2005).

Dada uma série temporal discreta unidimensional $\{x_1, \dots, x_i, \dots, x_N\}$, constroem-se séries temporais “sementes” (“coarse-grained”) consecutivas, $\{y^{(\tau)}\}$, determinada pelo fator escala, τ , de acordo com a equação:

$$y_j^{(\tau)} = \frac{1}{\tau \sum_{i=(j-1)\tau+1}^{j\tau} x_i}, 1 \leq j \leq N/\tau$$

Para a escala um, a série temporal $\{y^{(1)}\}$ é simplesmente a série temporal original. O comprimento de cada série temporal “semente” é igual ao comprimento da série temporal original dividido pelo fator escala, τ . Então, calcula-se uma medida de entropia para cada série temporal “semente”, gerando-se um gráfico em função do fator escala τ . Esse procedimento é o MSE.

A medida de entropia utilizada é a sample entropy (Samp-En) (Richman e Moorman, 2000). A $\text{SampEn}(m, r, N)$ corresponde ao logaritmo negativo natural da probabilidade condicional de que de que duas sequências similares para m pontos mantêm-se similares para o próximo ponto, onde auto-combinações não são incluídas no cálculo da probabilidade. Assim, um menor valor de SampEn também indica uma maior similaridade entre séries temporais.

As curvas geradas pela MSE são utilizadas para comparar a complexidade relativa das séries temporais normalizadas (mesma variância para a escala um) baseando-se na seguinte diretriz: (1) se para a maioria das escalas os valores de entropia são maiores para uma série temporal do que para a outra, a primeira é considerada mais complexa do que a segunda; (2) uma diminuição monotônica nos valores da entropia indica que o sinal original contém informação apenas na menor escala.

3.7 Análise estatística dos dados

Após avaliação da normalidade das variáveis dependentes analisadas, por meio do teste de aderência de Shapiro Wilks, receberão tratamento estatístico descritivo e inferencial por meio de análises de variância (ANOVA one-way) para comparação de cinco médias, após normalidade testada (teste de LEVENE). Serão consideradas as diferenças estatísticas com nível de significância igual a 5% ($\alpha = 0,05$). O tratamento estatístico será realizado no programa *Statistica v.8 (Statsoft Inc.)*.

A Análise de Cluster é uma técnica desenvolvida para identificar agrupamentos naturais que podem existir em uma população de interesse. Assim, ela permite alocar indivíduos em um grupo que contém outros indivíduos com características semelhantes. Essa alocação é feita a partir da análise das variáveis descritivas para cada indivíduo, que permite determinar as similaridades e as diferenças entre eles. O método permite, portanto, o descobrimento de informações ou padrões úteis, mas não óbvios, em grandes conjuntos de dados, e já foi utilizado para se estimar e comparar padrões cinemáticos e cinéticos de marcha entre indivíduos não diabéticos, diabéticos sem neuropatia e diabéticos neuropatas (Sawacha et al., 2010).

Após a classificação dos indivíduos em sem neuropatia, neuropatas leves, neuropatas moderados e neuropatas graves a partir da lógica fuzzy, a energia, frequência mediana dos músculos avaliados dos sujeitos diabéticos serão analisados juntamente com os padrões dos sujeitos do grupo controle, com base na Análise de Cluster, a fim de identificar as características biomecânicas dos agrupamentos desses sujeitos. Depois, verificar-se-á se os dados EMG foram capazes de distinguir os padrões EMG dos sujeitos com diferentes graus de neuropatia, ao se comparar os agrupamentos ou cluster realizados pela Análise de Cluster com a classificação obtida pela lógica fuzzy. Ou seja, verificar-se-á a distribuição dos sujeitos com os diferentes graus de neuropatia em cada agrupamento gerado pela Análise de Cluster.

Para a Análise de Cluster será desenvolvido um algoritmo linguagem matlab (Matlab 7.1, *Mathworks, Inc.*). que minimiza a soma das distâncias de cada objeto para o centroide do seu grupo (cluster) sobre todos os outros agrupamentos. O algoritmo move o objeto entre todos os possíveis agrupamentos até que a soma não possa mais ser diminuído. Nesse contexto, identificar-se-á o número ótimo de agrupamentos observando-se a informação referente a cada interação e o total da soma das distâncias. Essa soma pode variar a cada interação na medida em que o algoritmo realoca os pontos entre os agrupamentos e recomputa os centroides dos mesmos. A partir daí, será gerado um gráfico de silhueta para cada solução a fim de avaliar qual solução gera agrupamentos que são melhor separados do que as soluções anteriores. O valor da silhueta para cada ponto é uma medida do quão similar esse ponto é aos pontos do seu próprio agrupamento, em comparação aos pontos de outros agrupamentos. Essa medida varia de [1] (pontos muito distantes dos agrupamentos vizinhos) a [-1] (pontos que provavelmente foram alocados no agrupamento errado). O número de agrupamentos é aumentado para cada solução até que um agrupamento vazio seja criado. A solução que gera agrupamentos que são

melhor separados que soluções prévias é escolhido. As variáveis de entrada serão as variáveis calculadas pela análise de Wavelets (energia e frequência). O teste ANOVA será realizado a fim verificar as diferenças entre as variáveis dos diversos agrupamentos.

4. ETAPAS PARA O DESENVOLVIMENTO DO PROJETO - CRONOGRAMA

O projeto será desenvolvido em 4 anos:

Tabela 1 - Cronograma para as etapas do projeto.

PERÍODOS	2013 (2° sem)	2014 (1° sem)	2014 (2° sem)	2015 (1° e 2° sem)	2016 (1° e 2° sem)	2017 (1° sem)
ETAPAS						
Atualização da literatura	X	X	X	X	X	X
Instalação do equipamento EMG	X	X				
Treinamento da equipe no equipamento EMG		X	X			
Definição do protocolo de coleta de dados			X			
Experimento Piloto			X			
Coleta de dados				X	X	
Análise matemática das variáveis					X	X
Análise estatística multivariada					X	X
Interpretação e Discussão dos resultados					X	X
Elaboração do texto final e relatório final						X
Elaboração do artigo e textos de divulgação em congresso						X

5. FORMAÇÃO DA EQUIPE PARA O DESENVOLVIMENTO DO PROJETO

Coordenadora do Projeto

Profa. Dra. Isabel de Camargo N. Sacco
Responsável

Professora Associada MS5 FMUSP – Pesquisadora

Equipe do projeto

Eneida Yuri Suda
Reabilitação FMUSP

Doutoranda LABIMPH, Programa Ciências da

Aline Arcanjo Gomes
Reabilitação FMUSP

Doutoranda LABIMPH, Programa Ciências da

Cristina Dallemole Sartor

Pesquisadora colaboradora LABIMPH

Anice de Campos Pássaro

Apoio Técnico-científico

Graduanda Curso Fisioterapia da FMUSP

Estudante de Iniciação Científica

6. RISCOS E DIFICULDADES

Algumas características do protocolo experimental, assim como dos procedimentos metodológicos devem exercer alguma influência sobre os resultados que serão obtidos e desta forma, existirão limitações nas discussões subseqüentes. Tais limitações sempre existirão em qualquer procedimento experimental, cabe ao pesquisador delimitá-las e descrevê-las de forma a não produzir interpretações incorretas dos resultados discutidos.

Considerando a amostra experimental, alguns cuidados serão tomados quanto a seleção dos pacientes, tais como preservar a identidade dos sujeitos, respeitar suas limitações motoras, cuidados na clínica, funcional e biomecânica. Outras considerações referem-se ao diagnóstico clínico da neuropatia diabética, não sendo possível a realização de qualquer outro exame eletrofisiológico para este diagnóstico, em função da limitação de recursos tecnológicos do departamento nesta área. Ainda considerando a amostra, o n sugerido para o estudo da intervenção pode não ser atingido em função da demanda do serviço de saúde do Hospital Universitário e da ANAD, ou seja, caso nos meses que se propõe realizar a avaliação biomecânica dos diabéticos não houver tanta demanda de pacientes com neuropatia diabética, então pode não ser possível chegar no n proposto inicialmente.

As coletas experimentais da análise da marcha serão realizadas em ambiente de laboratório, dessa forma os voluntários serão avaliados em ambiente mais restritivo e diferenciado de uma condição habitualmente vivenciada por eles. Durante a avaliação do andar, será colocada uma situação ao paciente para que realize a marcha de forma espontânea até que se sinta seguro, no entanto, não podemos excluir que o novo ambiente, a nova tarefa imposta e talvez a falta de rotina em se andar descalço, exerça alguma influência sobre a marcha destes indivíduos. O conjunto destas alterações no padrão do movimento, devido ao ambiente ou ainda aos equipamentos de medição, denomina-se efeito retroativo. De forma a minimizar este efeito retroativo causado pelos equipamentos utilizados, os sujeitos serão submetidos a uma fase de habituação no local da coleta em que deverão caminhar por 5 minutos com os equipamentos necessários.

7. RESULTADOS ESPERADOS

7.1 Do ponto de vista científico

Os resultados e discussões obtidos serão divulgados em meios eletrônicos e impressos nacionais e internacionais, tornando-se públicos, através de periódicos científicos e anais de congressos. As perspectivas de produção e divulgação dos resultados encontrados serão:

- Duas teses de doutorado (Eneida Yuri Suda, Aline Arcanjo Gomes)
- Um trabalho de Iniciação Científica e de trabalho de conclusão de Curso
- Pelo menos 2 publicações em Congressos de Biomecânica e Fisioterapia
- Pelo menos 2 artigos em periódico internacional ISI – Qualis A1/A2

7.2 Do ponto de vista clínico

Em relação aos resultados esperados, buscar-se-á a curto prazo:

De uma forma geral, espera-se a obtenção de uma maior compreensão das estratégias de recrutamento muscular e coordenação motora intra membros inferiores durante a marcha, entendendo os momentos de maior demanda muscular e os momentos onde a população diabética poderá adotar uma estratégia diferenciada. O presente estudo tem a intenção de contribuir para um maior entendimento de como a progressão da doença diabetes mellitus acomete a biomecânica da marcha saudável.

Em relação aos resultados esperados, buscar-se-á a longo prazo:

Espera-se também que, ao entender a sinergia global das estratégias de recrutamento muscular e em que momentos ocorrem as principais alterações, poder-se-ia supor que tais estratégias devem ser previstas e prevenidas, de modo que a marcha diabética possa receber intervenções terapêuticas anteriormente a uma modificação definitiva de padrão.

Espera-se também, que futuros estudos sejam realizados que permitam a exploração de formas de tratamentos fisioterapêuticos, por meio de ensaios clínicos, que terão o objetivo de comprovar a eficiência de intervenções fisioterapêuticas nestas estratégias musculares e de coordenação adotadas por esta população que gerem um importante impacto na qualidade de vida. Pretende-se com o incentivo a estes ensaios clínicos, a incorporação, na terapêutica já adotada clinicamente a pacientes diabéticos, a fisioterapia direcionada aos déficits motores e biomecânicos detectados no presente estudo como um meio de se prevenir a formação de úlceras plantares e consequentes amputações.

7.3 Do ponto de vista da formação de recursos humanos

Pretende-se formar recursos humanos (doutores, graduando e pós doutor) engajados em pesquisa tecnológica e clínica nos anos de desenvolvimento do presente projeto, além de prepará-los para atuar em Instituições de Ensino Superior disseminando o conhecimento adquirido durante o curso de graduação e pós-graduação. Pretende-se:

- Formação de duas doutoras em Ciências da Reabilitação (Eneida Yuri Suda, Aline Arcanjo Gomes)
- Formação de uma Iniciação Científica e trabalho de conclusão de Curso

8. REFERÊNCIAS BIBLIOGRÁFICAS

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**Aprovação do Comitê de Ética em Pesquisa da Faculdade de Medicina da
Universidade de São Paulo (CEP-FMUSP)**

APROVAÇÃO

O Comitê de Ética em Pesquisa da Faculdade de Medicina da Universidade de São Paulo, em sessão de **29/05/2013**, **APROVOU** o Protocolo de Pesquisa nº **188/13** intitulado: **“PADRÕES DINÂMICOS DE RECRUTAMENTO MUSCULAR POR EMG DE ALTA DENSIDADE DE DIABÉTICOS EM DIFERENTES NÍVEIS DE SEVERIDADE DE NEUROPATIA, CLASSIFICADOS POR UM MODELO LINGÜÍSTICO FUZZY”** apresentado pelo Departamento de FISIOTERAPIA, FONOAUDIOLOGIA E TERAPIA OCUPACIONAL

Cabe ao pesquisador elaborar e apresentar ao CEP-FMUSP, os relatórios parciais e final sobre a pesquisa (Resolução do Conselho Nacional de Saúde nº 196, de 10/10/1996, inciso IX.2, letra "c").

Pesquisador (a) Responsável: Isabel de Camargo Neves Sacco

Pesquisador (a) Executante: Eneida Yuri Suda

CEP-FMUSP, 07 de Junho de 2013.

Prof. Dr. Paulo Eurípedes Marchiori
Vice-Coordenador
Comitê de Ética em Pesquisa

Comitê de Ética em Pesquisa da Faculdade de Medicina
e-mail: cep_fm@usp.br