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# Recurrence quantification analysis of force signals to assess neuromuscular fatigue in men and women

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Cyril Chatain – <u>cyril.chatain@univ-tln.fr</u> IAPS, Université de Toulon, 83130 La Garde, France Tel: +33 6 79 38 87 53 E-mail: cyril.chatain@univ-tln.fr Abstract: Recurrence quantification analysis (RQA) is a nonlinear method providing information on the temporal structure of time series. RQA has been extensively used to explore various noisy and nonstationary physiological signals. However, the application of RQA to force signals acquired during voluntary fatiguing contractions performed until exhaustion remain to be investigated. We aimed to explore the sensitivity of the percentage of determinism (DET), an RQA predictability measure, to detect changes of force signal complexity induced by fatigue and recovery. Changes in force signal complexity were compared between women and men to explore the ability of DET measures to detect different fatigue profiles. Nineteen women and nineteen men performed intermittent isometric contractions of knee extensors at 50% of maximal voluntary contraction (MVC) until exhaustion. Participants performed MVC before, during and after the fatiguing task to assess neuromuscular fatigue. Recovery measurements were performed three minutes after exhaustion. Particular attention has been given to the selection of the input parameters of RQA and to the influence of nonstationarity. A detailed methodology is provided to apply RQA to force signals. At the whole group level, complexity decreased with fatigue then increased after recovery. Greater fatigability of men was associated with a faster loss of complexity (i.e. faster increase of DET) of force signals. After recovery, complexity returned to baseline value only for women. These findings confirm that RQA is suited to explore force signal temporal structure and is able to reveal changes of complexity induced by fatigue and recovery by taking into account sex differences.

**Keywords:** Force signal fluctuations, recurrence quantification analysis, exercise-related fatigue, complexity, sex differences.

# 1) Introduction

Human performance is limited by fatigue which can be defined as a complex disabling symptom resulting from a myriad of physiological and psychological mechanisms [1]. Fatigue can be captured as a trait, in resting conditions, using individuals' self-reports. Fatigue can also be evaluated as a state, during a given task such as physical exercise. Exercise-related fatigue (which is also referred to as "fatigability" [2,3]) represents a major area of research, with applications in various fields including sports, industrial and clinical settings. Exercise-related fatigue can be assessed by the exercise-induced decrease in maximal voluntary force or power produced by a muscle or a muscle group, classically referring to the concept of "neuromuscular fatigue" [4,5]. In addition to its negative impact on maximal force capacity, neuromuscular fatigue also impairs motor output during submaximal isometric contractions. These impairments are usually assessed using measures quantifying the force fluctuations in terms of amplitude (i.e. standard deviation and/or coefficient of variation) [6–8]. More recently, it has been suggested that neuromuscular impairments could be evaluated by measures allowing to characterise the temporal structure of force signals (e.g. [9]).

Several methods, derived from dynamical systems, information, fractal, and statistical physics theories, have been introduced to evaluate the temporal structure of many physiological signals [10–14]. Clustered under the name of "measures of complexity", they provide complementary information to those obtained with traditional statistical indicators (e.g. coefficient of variation) or conventional linear measures (e.g. spectral analysis) [15,16]. For instance, measures of complexity of postural signals would be good candidate to distinguish fallers from non-fallers in older adults [17,18] or more sensitive for the assessment of neuromuscular fatigue than spectral analysis when applied to electromyographic (EMG) signals [15].

It has been suggested that the "complexity" of a physiological signal would reflect the richness and the adaptation capabilities of the physiological system that produced this signal [16,19,20]. Complexity of force signals is interpreted as the adaptative capacity of the neuromuscular system to internal and/or external perturbations induced by the motor task demand. Several studies showing a loss of complexity of force signals with the appearance of neuromuscular fatigue [9,21,22] have extended the theories of loss of physiological complexity initially devoted to aging or pathological context [11,16,23].

So far, entropy measures (quantifying information regarding the "regularity" of a time series) remain the most commonly used indicators to quantify the so-called complexity of force signals, whether or not in a context of neuromuscular fatigue [8,9,23–26]. However, it is well established that these measures can be, at least in some cases, biased by a characteristic often found in physiological signals that is nonstationarity (mainly low frequency trends) [27–29]. To overcome this limitation, a strategy allowing the detrending of force signals prior to entropy estimation has been recently proposed [29]. However, this approach was not suitable for the analysis of signals collected in the present study. Indeed, it was impossible to ensure stationarity through this strategy based on a statistical test for our dataset. Another method sometimes used to investigate the temporal structure of physiological signals, in terms of complexity, is the detrended fluctuation analysis (DFA), which is designed to quantify the correlations of time series. However, DFA is also sensitive to trends [30] and the interpretation of its results relies on stochastic models such as fractional Gaussian noises and fractional Brownian motions [31], which are not always compatible with experimental data.

Based on these arguments, we propose here to assess the complexity of force fluctuations using an alternative approach through the analysis of the recurrences of patterns extracted from the experimental signals. Using graphical representations of the patterns, the so-called recurrence quantification analysis (RQA) provides an efficient way to estimate dynamical invariants and complexity measures related to the underlying system generating the data (see [32] and references therein). RQA has many advantages such as its effectiveness for short and noisy time series and relevance for signals produced by nonlinear systems. It is also often described as a suitable tool for the investigation of nonstationary data [32,33].

Within the scope of neuromuscular fatigue, RQA has been widely applied to EMG signals [15,34–36]. One of the most used RQA measures is the percentage of determinism (DET) which reflects the predictability of a time series (or its regularity, in this context). RQA returns a DET value between 0 and 1 (generally expressed in percentage), which decreases with the irregularity of the time series, often related to complexity in the literature [32]. Therefore, the authors reporting an increase of DET for EMG signals with the appearance of neuromuscular fatigue confirmed the fatigue-induced loss of complexity of neuromuscular system [15,34–36]. Nevertheless, the ability of RQA to detect fatigue-induced changes in neuromuscular system complexity from force signals is currently unknown. The analysis of force signals seems particularly relevant in various clinical and applied contexts as force represents a more functional measure (whose dynamics is the gold standard to capture neuromuscular fatigue [5]) and is often easier, less costly and time-consuming to capture compared to EMG signals of all single muscles (e.g. vasti muscles and rectus femoris) composing a muscle group (e.g. knee extensors).

To our knowledge, only very few studies have used RQA to quantify the complexity of force signals. Kuznetsov and Riley [40] applied RQA to force signals acquired during finger flexions, with the aim to determine the impact of spatial resolution of visual feedback on signals complexity. Li *et al.* [37–39] also quantified the complexity of force signals recorded during grip contractions in different populations (i.e. healthy participants across various age and people with several neuromuscular disorders) through RQA, with the main aim to detect differences in force control strategies. However, in these studies force signals were collected during short contractions (i.e. 30-s duration), making difficult to assess the sensitivity of RQA to neuromuscular fatigue, especially because no conventional assessment of neuromuscular fatigue was performed (e.g. pre-post maximal voluntary contractions). Therefore, further studies are needed to understand the effect of neuromuscular fatigue on RQA measures obtained from force signals.

To enhance the assessment of the sensitivity of RQA to neuromuscular fatigue and its ability to detect inter-individual differences, we propose to compare two populations with well-known differences in terms of neuromuscular fatigue profiles, i.e. women vs. men. It has long been established that women are less fatigable than men under specific conditions, including submaximal isometric muscle contractions performed at the same relative intensity (for review, see [41]). We also propose to analyse recovery data to assess the ability of RQA to detect different muscle state changes (i.e. fatigue and recovery).

Accordingly, the aim of this study was to determine whether changes of neuromuscular system complexity could be detected by RQA applied to force signals, and to test the following assumptions: (i) that DET (obtained from RQA) would increase with neuromuscular fatigue then decrease after recovery; (ii) that the increase in DET with neuromuscular fatigue would be more important in men compared to women and (iii) that changes in DET values would correlate with traditional measures of neuromuscular fatigue and recovery (i.e. loss and recovery of maximal voluntary force).

# 2) Methods

# 2.1) Participants

Thirty-eight healthy young adults (nineteen women and nineteen men) volunteered to participate in this study and were kept naive to the protocol (Table 1). All subjects provided written informed consent to participate in the study, which was approved by a local ethic committee and conducted in accordance with the declaration of Helsinki.

	Men (n=19)	Women (n=19)	<i>p</i> value
Height (cm)	$180.3 \pm 5.1$	$164.4 \pm 4.5$	<0.001
Weight (kg)	$74.4 \pm 11.2$	$60.3 \pm 10.5$	<0.001
Age (years)	$23.0 \pm 2.7$	$22.2 \pm 3.2$	0.16
BMI (kg/m <sup>2</sup> )	$22.8 \pm 2.8$	$22.2. \pm 3.0$	0.51
Baseline MVC (N·m)	$349.7 \pm 57.3$	$223.7 \pm 45.0$	<0.001
Baseline DET (%)	$83.2 \pm 7.0$	$94.5 \pm 3.1$	<0.001

 Table 1. Participants information (mean  $\pm$  SD). BMI: Body mass index; MVC: Maximal voluntary contraction; DET:

 Percentage of determinism.

### 2.2) Experimental setup

Subjects sat in a custom-made chair with both hips and knees at  $90^{\circ}$  of flexion. A force sensor (F2712 200 daN, Celians MEIRI, France) was attached with an inextensible strap to the right leg ~ 3-4 cm above the malleoli of the ankle joint. A belt was secured firmly across the waist to avoid any extraneous movements during isometric contractions. Force data were collected at 2 kHz using BIOPAC MP150 (BIOPAC Systems, Inc., Santa Barbara, CA, USA) and Acqknowledge software (Version 4.1, BIOPAC Systems, Inc., Santa Barbara, CA, USA).

# 2.3) Protocol

The experimental sessions began with a standardized warm-up of the knee extensors including 4 min of intermittent isometric contractions performed at increasing force levels. Then, after 5 min of rest, participants performed at least three maximal voluntary contractions (MVCs) of  $\sim$  4-s duration interspaced by 1 min of recovery. Strong verbal encouragements were given during each MVC to ensure maximal volitional effort. If a gradual increase was observed during the first three attempts, additional MVCs were performed to ensure the reaching of the real MVC for each participant. Then, after 5 min of rest, participants began the fatiguing task.

During the fatiguing task participants were instructed to perform intermittent isometric contractions of the knee extensors (i.e. cycles of 8 s of contraction and 4 s of rest; Fig. 1) at 50% MVC until task failure. Verbal instructions were given throughout the fatiguing task to ensure that participants maintained the required target force level as accurately as possible. The force signal feedback was displayed on a screen (32" - 1920 x 1080 pixels) placed in front of the participants at a distance of ~ 120 cm. Since the spatial resolution of visual force feedback may impact the temporal structure of the signals [40], we used a fixed value of 11% MVC.cm<sup>-1</sup> for each participant. Task failure was defined as the first contraction at which the participant failed to reach the target force level (i.e. first contraction with no sample within or above the target force level during the full 8-s duration; Fig. 1).

Participants were asked to perform one MVC every five cycles (i.e. every minute) and immediately at task failure to evaluate the neuromuscular fatigue.

After task failure, participants were given 3 min of rest and were then asked to perform one MVC followed by one block of five submaximal isometric contractions of the knee extensors (i.e. 8-s duration interspaced by 4 s of rest).

#### 2.5) Data analysis

Endurance time and peak force during MVCs were calculated using Acqknowledge software. Endurance time was measured as the time separating the beginning of the first submaximal contraction and the end of the last block entirely completed (excluding time to perform the intermittent MVCs). Signals preprocessing and measures of complexity were computed with MATLAB R2019a (MathWorks, Natick, MA, USA). MVCs were compared at three time points: i) baseline (i.e. greatest MVC produced before the fatiguing task; Fig.1); ii) fatigue (i.e. MVC performed following the last block of submaximal contraction entirely completed; Fig.1) and iii) recovery (i.e. MVC performed after the 3 min of rest; Fig. 1). Complexity data of force signals were compared using the same time points: (i) baseline (i.e. first block of five submaximal contractions; Fig. 1); (ii) fatigue (i.e. last block of five submaximal contractions entirely completed; Fig. 1) and (iii) recovery (i.e. block of five submaximal contractions performed after 3 min of rest; Fig. 1). To allow optimal comparisons between men and women, changes of complexity and MVC were normalised to the baseline value (i.e. changes expressed in percentage of baseline value). Data were also expressed as rate of changes during the fatiguing task and the recovery period. For instance, changes for normalised MVC were expressed as rate of decrease during the fatiguing task and rate of increase during the recovery using the equations (1) and (2), respectively. The same analysis was performed for changes of normalised DET.

 $Rate of decrease for normalised MVC = \frac{\left(\frac{MVC_{fatigue} - MVC_{baseline}}{MVC_{baseline}}\right) \times 100}{Endurance time} (1)$   $Rate of increase for normalised MVC = \frac{\left(\frac{MVC_{recovery} - MVC_{fatigue}}{MVC_{baseline}}\right) \times 100}{Recovery time} (2)$ 



Figure 1. Force signal recorded during the experimental session in a representative male participant. The red dotted line represents the target force level which has to be maintained during submaximal contractions. The gray areas show the windows used for recurrence quantification analysis. MVC: Maximal voluntary contraction.

#### 2.5.1) Force signals preprocessing

For each submaximal contraction, the 1.5 first and last seconds were excluded (i.e. analyses were performed across 5-s centered window for each contraction; Fig. 1), then force signals were off-line filtered using  $6^{\text{th}}$  order zero-phase low-pass digital Butterworth filter with cut-off

frequency of 20 Hz and down-sampled at 500 Hz. Thereafter, the very low frequency trend of each signal was removed by means of a non-linear and data-driven method so-called Empirical Mode Decomposition (EMD) [42]. In short, EMD allows to decompose a signal into different components with locally defined frequency bands and therefore to limit the first-order nonstationarity of a signal after removing the lowest frequency component (also called residual; Fig. 2A and 2B). This procedure was not intended to rigorously guarantee the stationarity of signals (through statistical tests [29]) but was necessary to ensure that potential fatigue-induced changes in complexity are not mainly explained by very low-frequency trends (see below; part 2.5.2.2. Computation of complexity measures). Prior to RQA, the detrended signals were centered to have zero mean and scaled to have unit standard deviation.

#### 2.5.2) Recurrence quantification analysis

RQA is based on the reconstruction of the phase (or state) space (i.e. an abstract geometrical space whose coordinates represent the different states of the system under study) from a time series produced by a dynamical system (e.g. force signal produced by the neuromuscular system) following the Takens' delay embedding theorem [43–45] and using an embedding dimension m, a delay  $\tau$ , and the following equations:

$$y_i(m) = (x_i, x_{i+\tau}, x_{i+2\tau}, \dots, x_{i+(m-1)\tau}) (3)$$
  
$$n = N - (m-1)\tau (4)$$

where  $x_i$  is the *i*<sup>th</sup> point of the original time series,  $y_i(m)$  the *i*<sup>th</sup> sequence (also called time delay vector) of dimension *m*, *n* the number of sequences reconstructed in the phase space and *N* the length of the original time series.

#### 2.5.2.1) Construction of the recurrence plots

The second step of RQA consists in the construction of the recurrence plot (RP) introduced by Eckman *et al.* [46], which corresponds to a 2-dimensional representation of the recurrences of the dynamical system in the state space. The RP is directly derived from the  $n \ge n$  (see equation (4)) binary recurrence matrix defined by the following equation:

$$R_{i,j}^{m,\varepsilon} = \Theta(\varepsilon - \|y_i - y_j\|) (5)$$

where  $\Theta$  is the Heaviside function (i.e.  $\Theta(\mathbf{x}) = 0$  if  $\mathbf{x} < 0$  and  $\Theta(\mathbf{x}) = 1$  otherwise), || || is the Euclidean norm and  $\varepsilon$  is a defined threshold. The threshold  $\varepsilon$  allows to determine whether two delay vectors corresponding to instants *i* and *j* are close enough (or "neighbors") to define a recurrence point (i.e. a black point in the RP) at location (*i*,*j*). Two cases can then arise : i)  $||y_i - y_j|| \le \varepsilon$ , in this case the vectors  $y_i$  and  $y_j$  are considered neighbors resulting in a black point in the RP at coordinates (*i*,*j*); ii)  $||y_i - y_j|| \ge \varepsilon$ , in this case the vectors  $y_i$  and  $y_j$  are not considered neighbors resulting in a white point in the RP at coordinates (*i*,*j*). Both axes of the RP (conventionally upwards and rightwards) are the time axes. As defined here, a RP is always symmetric with the respect of a black main diagonal also called line of identity [32].

# 2.5.2.2) Computation of complexity measures

A simple visual inspection of the RP provides qualitative information on the temporal structure of the signal, which is naturally related to the underlying dynamics [45,46]. For instance, if the process studied is strongly nonstationary, the resulting RP will be characterized by an absence of recurrence points in the upper left and lower right corners. Here, this phenomenon was frequently observed for signals recorded after fatigue (Fig. 2), leading to an important heterogeneity of RPs between the different time points. This heterogeneity was characterized

by the clustering of numerous recurrence points close to the main diagonal line of the RP for signals displaying important low frequency trends (Fig. 2A and 2C). Since RQA measurements are based on the structures present in the RP, it was important to limit this heterogeneity to obtain results which correctly reflect changes in the dynamic of the time series and not produced by nonstationary trends. The EMD pre-processing, previously described in section 2.5.1. Force signals preprocessing, allowed us to remove these low-frequency trends before applying RQA.



Figure 2. (A) and (C) Force signals acquired during submaximal contractions performed after fatigue without removal of residuals (represented by the dotted lines) and their associated recurrence plots; (B) and (D) Preprocessed force signals of panel A and C, respectively, after removal of residuals and their associated recurrence plots. The duration of signals shown is 5 seconds.

Beyond a simple visual inspection, several measures have been introduced to quantify the structures of the RP. The first one is the recurrence rate (RR) or percentage of recurrence which corresponds to the density of recurrence points (i.e. black points) within the RP. The second classical measure derived from the RP is called percentage of determinism (DET) which corresponds to the percentage of recurrent points forming diagonal lines of at least length  $l_{min}$ . DET is computed using the equation (6):

$$DET = \frac{\sum_{l=l_{min}}^{n} {}^{lP(l)}}{\sum_{l=1}^{n} {}^{lP(l)}} \quad (6)$$

where P(l) is the histogram of diagonal lines of length l. DET is linked to the predictability of the system. For a deterministic system, the RP will have many long diagonals and few or none isolated points leading to an important value of DET. Inversely, for a system with random behavior, the RP will have none or very short diagonals leading to a low value of DET [32]. RQA provides many other output measures [32] but we focus here only on the DET outcome to quantify the regularity of force signals.

## 2.5.2.3) Selection of input parameters

The appropriate setting of the different input parameters (i.e. delay  $\tau$ , dimension *m* and the thresholds  $\varepsilon$  and  $l_{min}$ ) is crucial and particularly challenging when dealing with physiological data which may involve deterministic and stochastic features. Several methods have been proposed to optimise the selection of the input parameters.

#### 2.5.2.3.1) Delay $\tau$

A good estimation of delay  $\tau$  is important to perform reliable analysis. If the delay is too small, the successive time delay vectors will be strongly correlated and inversely, if the delay is too large the time delay vectors will be virtually independent [45]. Two methods are conventionally used to select the optimal delay  $\tau$ . The first one consists to select the time delay for which the autocorrelation function reach the value 1/e whereas the second one consists to choose the first local minimum of the average mutual information function [45,47]. Note that the average mutual information is sometimes preferred since it presents the advantage to consider the nonlinear dependencies not taken into account by the autocorrelation function. However, for most of our force signals these methods were not usable (i.e. significant variability for a same method between signals and/or inconsistent results between both methods for a given signal; Fig. 3).



Figure 3. (A) Example of pre-processed force signal of 5-s duration and its associated (B) autocorrelation and (C) average mutual information functions.

Therefore, the time delay  $\tau$  was selected using an approach previously used for the analysis of center of pressure signals presented in [17] (i.e. via the 2-dimensional phase space plot of  $x_i$  vs.

 $x_{i+\tau}$ ). Using this method, we found an acceptable time delay  $\tau = 20$  (i.e. corresponding to 40ms after down-sampling) allowing to ensure a reasonable deployment of the 2-dimensional phase space without the successive samples becoming completely independent (Fig. 4).



Figure 4. 2-dimensional phase plot from pre-processed force signal presented in Fig. 3A for (A)  $\tau = 1$  and (B)  $\tau = 20$ .

#### 2.5.2.3.2) Embedding dimension m

The optimal dimension *m* corresponds to the dimension in which the neighbors of each point (or time delay vector) within the reconstructed phase space can be considered as "real neighbors". Classically, this optimal dimension is selected using the global false nearest neighbor (FNN) algorithm [45,48]. This method is based on the computation of the number of neighbors of each delay vector within the phase space and observing how this number evolves when the embedding dimension *m* is increased. FNN algorithm relies on the ratio of distances separating two delay vectors in the dimension m and the dimension m-1. If this ratio is higher than a threshold (generally denoted  $R_{tol}$ ), the delay vectors are considered as "false neighbors". Figures 5B and 5C illustrate the projection of the phase space of a given force signal (Fig. 5A) from dimension 2 to dimension 3 and presents how two delay vectors can become "false neighbors" when the dimension is increased (i.e. the green and red points were close enough to be considered neighbors in dimension 2 but not in dimension 3). The optimal dimension mcorresponds to the dimension from which the percentage of false neighbors drop to 0. Results obtained with the FNN method were consistent for all force signals. Therefore, the optimal dimension m was set at 3 (Fig. 5D), in line with results reported in [37–39] from handgrip force signals.



Figure 5. (A) Example of pre-processed force signal of 5-s duration and its associated phase spaces for dimension m=2 in (B) and m=3 in (C). The green and red points in panels (B) and (C) represent two-time delay vectors considered as "false neighbors" when the 2-dimensional phase space is projected in dimension 3. (D) Effect of dimension on the percentage of false nearest neighbors for force signal presented in (A).

#### 2.5.2.3.3) Thresholds $\varepsilon$ and $l_{min}$

The threshold  $\varepsilon$  (sometimes called radius) defines the size of the neighborhood in the reconstructed phase space for which two points given by two delay vectors  $y_i$  and  $y_j$  can be considered close and will generate a recurrence point at location (i,j) (see equation (5)). Therefore, the choice of threshold  $\varepsilon$  is crucial. If  $\varepsilon$  is too large, almost all pairs of points of the phase space will be considered as neighbors, leading to many recurrence points in the RP. Inversely, if  $\varepsilon$  is too small, very few pairs of points will be neighbors leading to an absence of recurrence points in the RP. In both cases, the RP provides no information regarding the temporal structure of the time series under study. In contrast to dimension or delay, there is no generic method available in the literature to determine the optimal threshold  $\varepsilon$ . Several "rules of thumb" have been suggested, as set the  $\varepsilon$  according to a fixed RR [32]. Here, we made the choice to use a fixed RR. Indeed, since the different RQA measures are strongly dependent on the RR, which is dependent on the threshold  $\varepsilon$ , we used a variable radius to ensure a RR close to 1% for all force signals (i.e.  $1.18 \pm 0.06\%$ ).

Finally,  $l_{min}$  was set at 5 to prevent a threshold effect for the estimation of DET (i.e. avoid reaching the maximal value of 100%). To perform RQA computation we used the Cross Recurrence Plot Toolbox (version 5.22) developed by Marwan *et al.* [32,49]. Figure 6 presents examples of RPs obtained for a male and a female participant at different time points of the task (i.e. baseline, fatigue and recovery).



Figure 6. Recurrence plots generated from knee extensors force signals of 5-s duration acquired at baseline (A,D), after fatigue (B,E) and after recovery (C,F) for a male (A,B,C) and a female participant (D,E,F).

# 2.6) Statistical analysis

All statistical procedures were performed on Prism (Version 8.0.2, GraphPad Software, San Diego, CA, USA). For all variables, normality of distribution was visually inspected and checked using Shapiro-Wilk and D'Agostino-Pearson tests. Age, baseline MVC, baseline DET, anthropometric characteristics, endurance time, and rate of decrease and increase for normalised MVC and DET (i.e. expressed in percentage of baseline values) were compared between groups using unpaired t-test or Mann-Whitney test when conditions for the application of parametric test were not met. To detect effects of sex, time (baseline, fatigue, recovery) and any interactions for normalised MVC and normalised DET during the task, linear mixed models applying the Greenhouse-Geisser correction with one within group factor (time) and one between group factor (sex) were used followed by the Bonferroni multiple-comparison test. Linear mixed models were used because they allow to analyse repeated measures data with missing values. This statistical approach was particularly relevant for normalised MVC data since two samples were missing in the data set (i.e. two failed MVCs during experimental sessions). The same statistical procedure was performed to analyse normalised DET, but because linear mixed model needs normally distributed data, we decided to remove extreme values to ensure the normal distribution. To this end we used the ROUT method (i.e. robust regression followed by outlier identification) introduced in [50], which led to remove four extreme values of normalised DET (i.e. 3.5% of the data set). It is of note that statistical analyses were also performed with absolute values of MVC and DET. These analyses revealed similar results to those obtained with normalised values, but because it would be redundant to present the results in both absolute and relative values we reported here only results computed from normalised values. Relationships between traditional measures of neuromuscular fatigue and recovery (i.e. changes of normalised MVC) and changes in regularity of force signals (i.e. changes of normalised DET) and relationship between absolute values of baseline DET and baseline MVC were assessed using Pearson coefficient correlation when data were normally distributed and Spearman correlation coefficient for non-normal data. All data are presented as mean  $\pm$  SD within the text and figures. For all analyses, a significance level of p < 0.05 was used to identify statistical significance.

#### 3) Results

## 3.1) Traditional neuromuscular parameters

Men had higher baseline MVC compared to women  $(349.7 \pm 57.3 \text{ N} \cdot \text{m vs. } 223.7 \pm 45.0 \text{ N} \cdot \text{m},$  respectively; p < 0.001; Table 1). Consequently, the mean submaximal target force level to maintain during the fatiguing task was higher for men than women  $(174.9 \pm 28.6 \text{ N} \cdot \text{m vs. } 111.9 \pm 22.5 \text{ N} \cdot \text{m},$  respectively; p < 0.001). Endurance time was significantly longer for women than men  $(521 \pm 321 \text{ s vs. } 221 \pm 57 \text{ s, respectively; } p < 0.001$ ; Fig. 7).



Figure 7. Influence of sex on endurance time. Color symbols represent the individual data (n = 19 for each group). The horizontal black lines represent mean ± SD. \*: significant difference.

Main effect of time was found for normalised MVC ( $F_{(1.8,62.3)} = 598.5$ ; p < 0.001) with no significant main effect of group ( $F_{(1, 36)} = 0.16$ ; p = 0.69) or interaction effect (time × group;  $F_{(2, 70)} = 0.34$ ; p = 0.72). When considering all participants, normalised MVC decreased significantly with fatigue ( $\Delta 42.8 \pm 6.9\%$  of baseline value; p < 0.001) then increased significantly after recovery ( $\Delta 17.8 \pm 7.0\%$  of baseline value; p < 0.001) but remained lower than baseline (p < 0.001).

As the decrease of normalised MVC was similar for both groups and considering the shorter endurance time for men, the rate of decrease for normalised MVC during the fatiguing task was significantly greater for men than women (-0.21 ± 0.08 % of baseline value.s<sup>-1</sup> vs. -0.11 ± 0.05 % of baseline value.s<sup>-1</sup>, respectively; p < 0.001; Fig. 8A). However, the rate of increase for normalised MVC during recovery was not significantly different between men and women (0.09 ± 0.03 % of baseline value.s<sup>-1</sup> vs. 0.11 ± 0.05 % of baseline value.s<sup>-1</sup>, respectively; p = 0.48; Fig. 8B).

#### 3.2) Recurrence quantification analysis

At baseline, DET was significantly lower for men than women  $(83.2 \pm 7.0\% \text{ vs. } 94.5 \pm 3.1\%; p < 0.001;$  Table 1).

Effect of time ( $F_{(1.3,43.7)} = 35.68$ ; p < 0.001), group ( $F_{(1, 36)} = 23.24$ ; p < 0.001) and interaction (time × group;  $F_{(2, 68)} = 19.49$ ; p < 0.001) were found for normalised DET. When considering all participants, normalised DET increased significantly with fatigue ( $\Delta 9.6 \pm 10.4\%$  of baseline value; p < 0.001) then decreased significantly after recovery ( $\Delta 2.2 \pm 3.5\%$  of baseline value; p = 0.002) but remained higher than baseline (p < 0.001). After the fatiguing task, the significant increase of normalised DET was more important for men ( $\Delta 15.5 \pm 11.2\%$  of baseline value) than for women ( $\Delta 2.9 \pm 2.2\%$  of baseline value) (p < 0.001). After recovery, normalised DET decreased significant difference between recovery and baseline; p = 0.17). However, the decrease of normalised DET after recovery was not significant for men ( $\Delta 2.4 \pm 4.5\%$  of baseline value; p = 0.09).

Considering the shorter endurance time for men compared to women, the rate of increase for normalised DET during the fatiguing task was significantly greater for men than women (0.08  $\pm$  0.06 % of baseline value.s<sup>-1</sup> vs. 0.02  $\pm$  0.04 % of baseline value.s<sup>-1</sup>, respectively; p < 0.001; Fig. 8C). However, the rate of decrease for normalised DET during recovery was not significantly different between men and women (-0.01  $\pm$  0.03 % of baseline value.s<sup>-1</sup> vs. -0.01  $\pm$  0.01 % of baseline value.s<sup>-1</sup>, respectively; p = 0.93; Fig. 8D).



Figure 8. Influence of sex on (A) rate of decrease for normalised MVC from baseline to fatigue; (B) rate of increase for normalised MVC during 3 min of recovery; (C) rate of increase for normalised DET from baseline to fatigue and (D) on rate of decrease for normalised DET during 3 min of recovery. Color symbols represent the individual data. Data are presented as mean ± SD (n = 19 for each group except for women group in panel B where n = 17). \*: p<0.001: significant difference.

#### 3.3) Correlations between complexity and traditional neuromuscular parameters

When considering all participants, baseline values of MVC and DET (expressed in absolute values) were significantly correlated (R = -0.36; p = 0.028; Fig. 9A). The rate of decrease for normalised MVC during the fatiguing task was significantly correlated to the rate of increase for normalised DET (R = -0.76; p < 0.001; Fig. 9B) whereas the rate of increase for normalised MVC during recovery was not correlated to the rate of decrease normalised for DET (R = 0.12; p = 0.49; Fig. 9C).

When correlations were performed separately for men and women, baseline values of MVC and DET were not significantly correlated, for both men (R = 0.32; p = 0.18) and women (R = -0.17; p = 0.48). The rate of decrease for normalised MVC during the fatiguing task was significantly correlated to the rate of increase for normalised DET, for men (R = -0.64; p = 0.003) and women (R = -0.62; p < 0.01). For both men and women, the rate of increase for normalised MVC during recovery was not correlated to the rate of decrease for normalised DET (R = 0.28; p = 0.24 and R = 0.12; p = 0.66, respectively).



Figure 9. Scatterplot of (A) absolute baseline maximal voluntary contraction (MVC) as function of absolute baseline percentage of determinism (DET), (B) rate of decrease for normalised MVC during the fatiguing task as function of rate of increase for normalised DET and (C) rate of increase for normalised MVC during recovery as function of rate of decrease for normalised DET. Black lines represent the normalised slopes for all participants (n = 38 except in panel C where n = 36).

# 4) Discussion

Although RQA has been applied to many physiological signals, to the best of our knowledge, its application to force signals in a context of neuromuscular fatigue has never been studied. The major novel finding of the present study is that DET obtained from RQA is sensitive to changes of force signal complexity induced by fatigue and recovery. Our results also highlight that DET allows to discern sex differences regarding these changes. The significant relationship between the rate of decrease in complexity and a measure of fatigability supports that the former computed in the context of submaximal contractions can be considered as a relevant tool for the quantification of neuromuscular fatigue.

# 4.1) RQA detects muscle fatigue and recovery

As hypothesized, DET measure from RQA increased significantly with the appearance of neuromuscular fatigue. This result reflects a fatigue-induced loss of complexity of force signals, corroborating previous results of the literature obtained with others indicators of complexity (i.e. entropy and fractal measures) [9,21,22]. This finding is particularly relevant to extend the "toolkit" of metrics available to assess changes of force signal complexity. Changes of DET with the appearance of neuromuscular fatigue has been largely demonstrated for EMG signals [15,34–36]. It has been suggested that this result is mainly due to the synchronization of motor units caused by fatigue [15]. Since this synchronization also impacts the fluctuations of force signals [51,52], it seems very likely that such a mechanism is involved in the increase of DET of force signals reported here.

The increase of DET with the appearance of neuromuscular fatigue was previously suggested by Li *et al.* in [39] for force signals acquired during brief handgrip contractions (i.e. by dividing the contractions of 30-s duration performed at 70% of maximal voluntary force in 5-s windows). However, this result needed to be confirmed for several reasons: (i) the increase of DET was not reported in other studies conducted by the same research group using identical protocols [37,38], which is probably explained by the differences in the populations studied and/or the sample size; (ii) the force signals analysed were obtained during muscle contractions which were not conducted until task failure, inducing different levels of fatigue between participants and (iii) no traditional fatigue assessments were performed, making impossible to quantify the level of fatigue induced by the muscle contractions.

The significant relationship between an index of fatigability (i.e. rate of decrease for normalised MVC) and an index of loss of complexity (i.e. rate of increase for normalised DET) supports that RQA applied to force signals might be an interesting new indicator to assess neuromuscular fatigue. Nevertheless, it is important to note that rate of decrease for normalised MVC reflects changes of maximal force capacities whereas the DET from RQA was obtained from signals acquired during submaximal contractions. Future studies involving EMG measurements could investigate the relationship between the rate of decrease of force signal complexity and the rate of increase of the root mean square of EMG signals, a commonly used index for neuromuscular fatigue quantification [53].

Beyond its capacity to detect fatigue, the DET measure is sensitive to recovery. At the whole group level, the short period of rest allowed a significant recovery of the maximal voluntary force capacities concomitantly to a significant decrease of DET (index of complexity recovery). Although our results do not indicate a significant relationship between the rate of decrease for normalised DET and the rate of increase for normalised MVC, this finding suggests that DET

computed from force signals is a sensitive index to detect several state changes of the neuromuscular system (i.e. fatigue and recovery).

# 4.2) RQA detects sex differences

As expected, endurance time was significantly more important for women than men  $(521 \pm 321$  s vs.  $221 \pm 57$  s). Additionally, rate of decrease for normalised MVC force was significantly greater for men than women. These results are in line with numerous studies showing that women are less fatigable than men during submaximal isometric muscle contractions performed at the same relative intensity [41].

An important finding is related to the sex differences regarding the complexity of force signals. Men showed a greater complexity (revealed by a lower value of DET) of force signals than women at the beginning of the fatiguing task (i.e. baseline or without fatigue). Similar results were previously reported in studies assessing complexity by means of sample entropy [24,54]. Our results show a significant negative relationship between MVC and DET obtained at baseline. Although this relationship was not confirmed when computed on both groups separately (potentially explained by the reduction of samples size), this finding suggests that the differences of complexity of force signals between men and women are likely explained by the absolute force level produced by the participants (which was significantly greater for men than women). Another explanation could concern the inverted U-shaped relationship between the level of force generated (i.e. expressed in percentage of MVC) and the complexity of force signals [8,24,55]. It has been shown that there is a range of force level (~ 40-60% MVC) for which the complexity of force signals would be optimal. However, the results presented in the aforementioned studies were obtained from both men and women and concerned upper limb muscle groups. In view of the potential differences in the physiological characteristics of neuromuscular system between men and women (e.g. muscle fiber type) [41], it is not unlikely that this relationship is not perfectly similar for both sexes. For instance, it would be possible that the optimal force complexity will be found at ~ 50% MVC for men and at ~ 70% MVC for women, which could explain the differences of baseline complexity reported here. Further studies using several measures of complexity are needed to increase our understanding regarding the sex and muscle group influences on the relationship between force levels and the complexity of force signals.

Another new finding from the current study is the influence of sex on the changes in complexity of force signals induced by fatigue and recovery. Our data show a greater rate of increase for normalised DET for men than for women during the fatiguing task, indicating a faster loss of complexity for men. Regarding recovery, no significant difference between groups was reported for the rate of decrease for normalised DET, indicating a similar rate of recovery of complexity between men and women. Nevertheless, after recovery the decrease of normalised DET was significant only for women and can be considered as complete since no significant difference was reported between the value of DET obtained after recovery and the baseline value. These differences between sexes are probably due to the several factors explaining the differences of fatigability between men and women (e.g. types of fibers, oxidative capacities, muscle perfusion, voluntary activation) [41]. Further studies are needed to clarify the underlying neuromuscular mechanisms behind these differences.

# 4.3) Influence of input parameters

As mentioned above, the selection of appropriate input parameters of RQA is not straightforward, especially for the analysis of physiological signals. Here, this selection was

particularly difficult for the time delay  $\tau$  by using traditional methods presented in the literature (i.e. autocorrelation and mutual information functions). Considering that these methods were not suitable for the analysis of our signals, it was more appropriate to use another approach based on a visual inspection of 2-dimensional phase spaces. The use of this approach was motivated for the reason that it was previously used for the analysis of postural signals [17], which exhibit similar features to force signals obtained in our study. Nevertheless, since the visual nature of this method represents an important limit, we derived RQA measures with a range of time delay (10-30) to confirm the qualitative consistency of our results. Since all input parameters have an influence on the measures derived from RQA, we used a range of values for each parameter (dimension m = 2-4, RR = 1-10,  $l_{min} = 2-5$ ). Using alternative values of these parameters within these ranges, no qualitative changes were observed regarding similar statistical analyses. Note that for some parameters the results of the DET were not usable. For instance, for several RR and  $l_{min}$  values (i.e. greater than 2% and 3 respectively) we commonly observed a sort of threshold effect for DET values (i.e. values close to the maximal value of 100%). This phenomenon had the effect of masking any change in complexity, whether under the influence of sex, fatigue or recovery.

## 4.4) Limitations

A first limitation is that all experimental sessions were performed on the right leg. The dominant lower limb was not recorded, which could slightly impact the changes of the dynamic of force fluctuations and differences reported between sexes (especially in the case of significant difference regarding the ratio dominant/non-dominant limb between men and women).

Moreover, we assessed the sensitivity of RQA to fatigue using only two time points (i.e. baseline and fatigue). Indeed, the short endurance time for some male participants did not allow presenting the full kinetic of DET and MVC changes along the fatiguing protocol. To this end, future studies should use protocols allowing longer tests duration for all participants, either by using lower target force level and/or changing the duty cycle (i.e. ratio contraction / relaxation) if intermittent contractions are used.

# 5) Conclusion

This study provides evidence that RQA is an interesting tool for detecting changes in the complexity of force signals induced by fatigue and recovery. When the selection of input parameters is performed properly, this method can expand the toolkit of measures used to quantify the complexity of force signals. Future studies regarding the changes of complexity of the neuromuscular system with the appearance of neuromuscular fatigue or after recovery should consider the differences between sex reported in the present study. In practice, DET computed from force signals recorded during submaximal contractions might be an alternative to quantify neuromuscular fatigue, which may be relevant in various clinical and applied settings.

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