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Motor unit synchronization and firing rate correlate with the fractal dimension of the surface EMG: A validation study

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ABSTRACT

The fractal dimension (FD) of the surface electromyographic (EMG) signal has been reported to be influenced by changes in the firing rate and synchronization of motor units. The purpose of this study was to validate these relations during experimental signals.

Thirteen healthy subjects (12 men and 1 woman) performed an isometric knee extension at 5 % of their maximal voluntary contraction for 300 s. Intramuscular and surface EMG signal were recorded concurrently from the vastus medialis obliquus. Synchronization and firing rate were calculated from the decomposed intramuscular EMG signal, while FD was estimated using the box-counting method. The first and last $50 \, s$ of contractions were considered during the correlation analyses.

FD was negatively related to the level of motor unit synchronization ($r_s=-0.30$; p<0.05) and positively correlated with firing rate ($r_s=0.25$; p<0.01) when all data were pooled. FD was correlated with firing rate only during the initial 50 s of contraction ($r_s=0.52$; p<0.001).

FD of the sEMG signal is a parameter mostly related to the firing rate when fatigue does not develop and may be considered as an index of performance fatigability during sustained or at the end of prolonged contractions at very low forces. Indeed, FD cannot be considered as an exclusive index of motor unit synchronization during fatiguing contractions, but rather as largely related to central factors.

1. Introduction

Changes in the surface electromyogram (sEMG) during a fatiguing task may be detected using non-linear analysis, which provides a measure of signal complexity. Recent works suggested that non-linear parameters such as signal entropy or fractal dimension (FD) are highly sensitive to hidden rhythms on the sEMG signal during fatigue and thus may be used as indirect fatigability indices [1–3]. The fractal's theory refers to the discovery of Benoit Mandelbrot [4]: 'an object or a signal which can be split into parts, each of which is a reduced-size copy of the

whole, might be defined as fractal and this property is called self-similarity'. The determination of the FD of a signal gives a quantitative indication of its geometrical complexity, and is also related to the degree of interference, which is inversely related to the 'smoothness' of the signal [5,6]. One of the most popular methods to calculate the FD of the sEMG signal is the box-counting [3,6,7] and a recent investigation showed a good reliability of FD during isometric contractions in the biceps brachii [8]. Initially, FD was used to characterize levels of muscle activation [7,9–11] and patterns of motor unit (MU) recruitment [3,12]. Later, a decrease in FD was associated to aging [13,14], disease [15,16]

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and fatigue [17].

Previous publications suggested that a decay of FD during isometric fatiguing contractions was associated to an increase in MU synchronization, as expression of the central nervous system (CNS) adaptation to fatigue [5,14,18]. MU synchronization is the tendency of separate MUs to discharge near simultaneously (within 1-5 ms of each other) more often that would be expected by chance [19]. Common synaptic input delivered by branched neurons at the level of the spinal cord produces correlated discharges of action potentials by motoneurons [20]. The effect on MU activity is quantified as MU synchronization and is traditionally measured invasively with needle or fine-wire electrodes (intramuscular EMG, iEMG), by time- and frequency-domain analyses of the discharge times of pairs of MUs [21]. Although technically challenging, the measurement of MU synchronization represents one of the only few techniques (besides sEMG decomposition) that allows a functional examination of the intact CNS during voluntary contractions. However, only a very small MU population of the active muscle is typically examined when estimating MU synchronization by needle EMG. More representative indices to quantify MU synchronization from a larger population of active MUs have been proposed using sEMG (e.g., [22,23]). Although these methods have demonstrated sensitivity to MU synchronization, they are highly dependent on muscle fiber conduction velocity (CV), and this is a major limitation since MU synchronization level and CV often change in parallel [24]. In contrast, studies on FD during simulations have shown low dependence on CV [5,25]. Because of the potential advantages of using FD of the sEMG for quantifying MU synchronization, there have been attempts to provide direct evidence of the association between the decay of FD during isometric fatiguing contractions and increase in MU synchronization, both during simulated and voluntary contractions. In particular, Mesin and colleagues [5] found that FD was mostly related to the level of synchronization and least related to the changes of muscle fiber CV. Later, Mesin et al. [25] evidenced during simulated contractions, the existence of an inverse relationship between FD and MU synchronization and a positive relation with MU firing rate (FR). Hence, the purpose of this study was to investigate in vivo whether the FD of the sEMG signal is related to MU synchronization and FR. This methodology was compared with findings obtained from single muscle fiber recordings and cross-correlation analysis.

2. Materials and methods

2.1. Participants

Thirteen healthy recreationally active subjects (12 men and 1 woman) with no history of neurological conditions and free of medication volunteered to participate in the study (mean \pm SD, age: 32 \pm 9 years; stature: 179 \pm 8 cm; body mass: 76 \pm 8 kg). All participants signed a written informed consent form before participation in the experiments. The study was approved by the local ethics committee of the IRCCS Policlinico San Matteo, Pavia (Italy), prot 2022-3.11-143. All procedures were conducted according to the Declaration of Helsinki.

2.2. Experimental procedure

Participants seated on an isokinetic dynamometer (Technogym, Cesena, Italy) equipped with a load cell (Model TF022, CCT Transducers, Turin, Italy) with the hip and distal thigh firmly strapped to the chair, the lower right leg secured to the dynamometer lever arm above the lateral malleolus, and the rotational axis of the dynamometer aligned with the right lateral femoral epicondyle. The knee was flexed at 90°. The reference maximal voluntary contraction (MVC_pre) for the definition of the submaximal force level was selected as the highest value of two isometric knee extensions for vastus medialis obliquus (VMO) over a period of 5 s, separated by 2 min of rest. Participants were instructed to increase the force up to their maximum and to hold it as

steady as possible. After the MVC measure, intramuscular and surface electrodes for EMG signal detection were mounted on the right VMO, as described below. The knee flexion force was displayed on a computer monitor facing the subject. The subjects were asked to sustain the force level on a target that was visually presented as a horizontal line at the midlevel of the screen. The subjects performed an isometric knee extension at 5 % MVC for 300 s and immediately after a third MVC (MVC_post). The amount of performance fatigability was quantified as the decline in MVC after the contraction [26].

2.3. EMG recordings

Surface and intramuscular EMG signals were recorded concurrently. The iEMG signal was used to detect the discharge times of individual MU, and this information was used to calculate the MU synchronization index (Synch Index), as described below.

To determine the optimal electrode placement from the right VMO, the position of the innervation zone was determined with the assistance of a dry linear electrode array of 16 electrodes (SA 16/5, OTBioelettronica, Turin, Italy) and marked on the skin [27]. sEMG signals were recorded by two couples of bipolar electrodes (22×30 mm; Kendall Neonatal ECG electrodes, OTBioelettronica) at about 1 cm distal to the innervation zone, detected in monopolar configuration and amplified (EMG amplifier, EMG-USB2+; OTBioelettronica, bandwidth 10–500 Hz; gain 1000), sampled at 10 kHz, and stored after 12-bit A/D conversion.

Single-motor unit action potentials were recorded with a pair of stainless-steel wires (diameter: 0.11 mm; Spes Medica, Genoa, Italy) inserted with 25-gauge hypodermic needles, at about half of the distance between the two coupes of sEMG electrodes (Fig. 1). Needles were removed, and the wire bundle remained lodged inside the muscle. The wires provided bipolar signals which were amplified (EMG-USB2+, OTBioelettronica), band-pass filtered (500 Hz–5 kHz; gain: 2000), sampled at 10 kHz, and stored on a computer. The position of the wires was slightly adjusted before starting the recordings, and when the signal quality was poor, which occurred rarely, new wires were inserted. Force was sampled at 10 kHz and low pass filtered (cut-off frequency 5 Hz). The ground electrode was mounted at the right ankle.

2.4. Single motor unit analysis

iEMG signals were decomposed into MU discharge trains using an interactive decomposition algorithm, EMGLAB [28] which includes a user interface for manually editing and verifying the accuracy of the spike trains. The MU analysis was performed according to the method described in [29]. After automated discrimination using the Montreal algorithm (MTL, [30]) during signal epochs of 10 s was accomplished, each MU spike train was manually edited and examined for any discrimination issues by a skilled operator. Complex superimpositions [31,32] were solved manually by a trained operator, when the automatic template-matching phase failed. The residual of the iEMG signal (i.e., the signal left after the templates of the discovered MU action potentials (MUAPs) have been removed from the interferential signal) was used to detect decomposition errors. The decomposition tool allowed for visual verification of the accuracy of the identification by comparing the original EMG signal to the residual after subtracting the detected MUAPs. This approach was performed until the residual matched the baseline noise level of the signal. It was considered that decomposition errors were present, when the residual signal's strength was not at noise level, and these cases were manually verified.

2.5. Synchronization index

The Synch Index was computed using the method described in [33] by measuring only the first order forward and backward recurrence times (the nearest forward and backward firing times) of the alternate MU with respect to each firing of a reference MU. The reference MU was

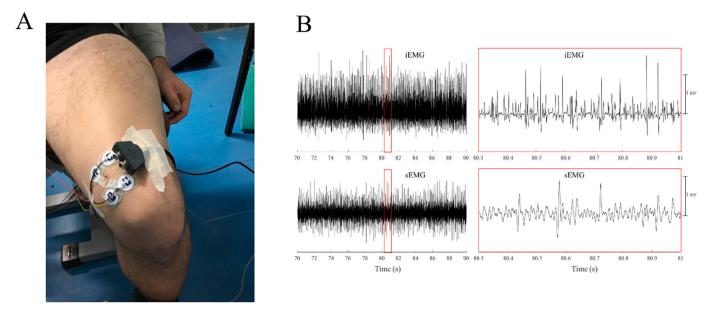


Fig. 1. Two pairs of bipolar surface EMG electrodes were positioned on the belly of the vastus medialis obliquus about 1 cm away from the innervation zone. The fine-wire intramuscular EMG electrode was inserted at the middle of the distance between the two pairs of surface electrodes (A). Examples of intramuscular (iEMG) and surface EMG (sEMG) signals from a representative participant (B).

chosen as the one whose MUAP train had the least number of firings.

For each pair of MUs, the histogram of minimum delay between each firing and the temporal closer firing of the other MU was computed using bin-width of 1-ms. The central bin (centered in T=0) included in this way the pair of firings with delay lower than 0.5 ms, thus synchronized (Fig. 2).

The ratio between the height of the central bin and the average height of the other bins, normalized as a percentage represented the Synch Index. In this way, if the probability of having synchronized firings was higher than the probability of having a uniform random distribution, the Synch Index would have been larger.

2.6. Fractal dimension

FD was estimated using the box-counting method, as previously reported [3,8]. Briefly, a grid of square boxes is used to cover the signal, and the number of boxes that the sEMG waveform passes through is counted (Fig. 3). When the box size decreases, the number of the boxes

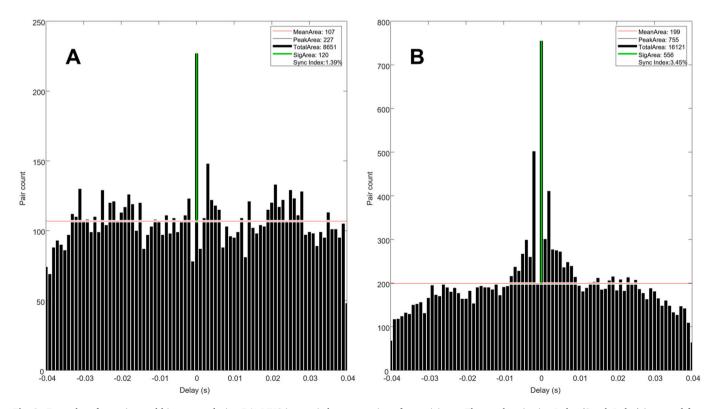


Fig. 2. Examples of cross-interval histograms during 5 % MVC isometric knee extension of a participant. The synchronization Index (Synch Index) increased from 1.39 % to 3.45 %, during the first (A) and last 50 s (B) of the contraction.

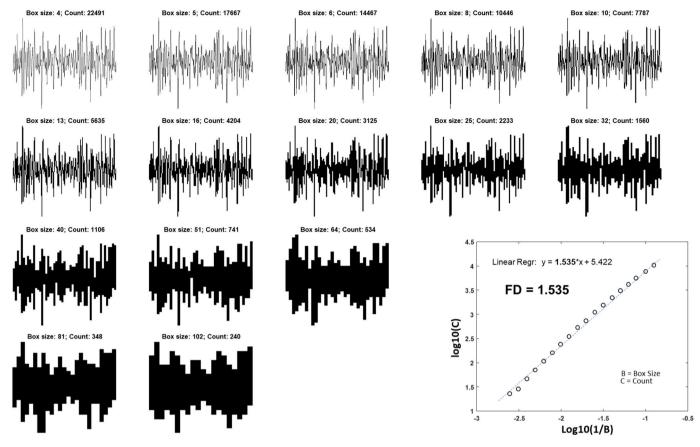


Fig. 3. Graphical representation of the box-counting algorithm used to determine the fractal dimension of the surface EMG waveform. The total number of boxes entered by the waveform (shaded region) are counted as the size of the overlying grid successively increased in size. The fractal dimension is computed as the slope of the regression line of the logarithm of box count versus box size.

that are counted will increase exponentially. The range of box size was restricted in order to avoid saturation for high and low value of size [3].

The box size was fixed to 13 steps equally spaced in logarithmic scale, with the smallest box equal to 1/128th of a second and the largest box equal to 1/8th of a second. The vertical side of the boxes was normalized to the range of the signal during epochs of 1 s and divided in the same number of boxes. However, by plotting the logarithm of the number of boxes counted vs. the logarithm of the inverse of the box size, the exponential relationship becomes linear. The slope of the interpolation line (estimated using the least mean squared procedure) is the fractal dimension (FD) [34]. Therefore, the following expression defines the FD:

$$FD = \frac{logN}{log\frac{1}{I}}$$

where N is the number of boxes required to cover the signal and L is the box size, with the ratio indicating the slope of the interpolation line. The FD of a continuous-time signal takes values between 1 (smooth signals) and 2 (stochastic or deterministic signals filling the whole space) [5].

2.7. Statistics

Linear regression over time was applied to FD to extract initial values and to the mean of FD to investigate its behavior along the entire 300 s sEMG signal. A Shapiro–Wilk test revealed that the variable distributions deviated from normality; consequently, the analyses were conducted using nonparametric statistical indicators.

Differences in the considered variables between the first (t1) and last 50 s (t2) of the contractions, were analyzed using the Wilcoxon signed-rank test. The Spearman's correlation coefficient (r_s), to assess the degree

of correlation between FD and the Synch Index, was computed. The statistical significance was set at $\alpha=0.05$. All statistical analyses were carried out with SPSS version 26.0 (SPSS Inc., Chicago, IL, USA), and the results are reported as median and interquartile range [IQR].

3. Results

The median MVC_pre was 568.8 [196.1] Nm, while the MVC_post was 362.9 [186.3] Nm. Consequently, the maximal strength post fatigue was 36.2 % significantly lower than the value at rest (p < 0.01).

A linear relation with time was observed for FD, with a continuous reduction during the complete signal of $-5.066\text{E-}05~(\text{R}^2=0.595,~p<0.001)$. Since the behavior of FD was approximately linear, and in order to compare a non-fatigued state with a period where the VMO was most probably undergoing fatigue, only the beginning and end of the signals, t1 and t2, respectively, were considered for the correlation analysis.

Full decomposition of the iEMG signals revealed 389 MUs at t1, and 456 MUs at t2. Four subjects were excluded from further analysis due to poor decomposition accuracy (i.e., too many errors in solving superimpositions). On average, across the 9 subjects during the five 10s signal epochs, 77.8 \pm 14.6 MUs at t1, and 91.2 \pm 4.8 MUs at t2, were decomposed.

The median MU FR was 9.3 [4.0] at t1 and 11.0 [9.0] at t2 of the 5 % MVC isometric knee extension with no significant differences between t1 and t2 (p = 0.21). The median frequency of each individual MU was 8.22 [2.07] Hz at t1, and 7.92 [3.25] Hz at t2, with no significant differences between t1 and t2 (p = 0.96).

The Synch Index between t1 and t2 increased from 0.33 [1.07] to 1.39 [2.21] (p = 0.002), whereas FD decreased from 1.535 [0.022] to 1.522 [0.032] (p < 0.001). Significant low to moderate correlations

were obtained between FD and Synch Index (Fig. 4), and between FD and FR (Fig. 5) when all data were pooled together (p < 0.05). Results of the correlation analysis are presented in Table 1.

4. Discussion

This study showed that during a low-level isometric knee flexion sustained for 5 min, the MVC declined significatively, suggesting the presence of performance fatigability [35]. In literature, three physiological factors have been identified as being responsible for the changes in the sEMG signal due to the onset of fatigability: (1) a decay in muscle fibers CV [36,37]; (2) an increase of the level of MUs synchronization by the CNS [38] and (3) a reduction of the recruitment threshold of MUs [39]. Several studies suggested that FD of the sEMG signal was not associated with changes in muscle fiber CV, but rather to central factors, such as MU synchronization [5] or FR [12]. The findings related to the Synch Index, evaluated by the standard technique based on single fiber recordings [33], suggested that a significant increase in MU synchronization was present between the initial and final 50 s. However, the median MU FR showed an increase between t1 and t2, though not significant. A similar result was described also in the study of Pascoe et al. [40] where the mean discharge rate did not change significantly during long-duration submaximal isometric contractions.

The FD of the sEMG signal showed a moderate inverse correlation with a direct measure of MU synchronization and a positive weak correlation with the FR when all the data were pooled together, suggesting that the geometrical complexity of the signal was influenced both by FR and the synchronization level. Interestingly, at t1 a much stronger positive correlation between FD and the FR was found (Fig. 5), while FD and synchronization level were not correlated. It may be hypothesized on the one hand that the initial 50 s of a 5 % MVC contraction were not fatiguing, thus with low levels of synchronization, and on the other hand, that the signal was mostly characterized by recruitment of MUs with high FR. On the contrary, we may hypothesize that the absence of correlations toward the end of the contraction, may be caused by three factors: (1) the signals were more similar to each other, with a fatiguemediated loss of complexity [34]; (2) concurrently in some subjects MU synchronization increased, so that the signal was also characterized with changes in the shape of the MUAPs, along with superimpositions that reduced FD and (3) an increase in the median MU FR. Similarly, Cashaback et al. [41] suggested that the fatigue-mediated reduction in

signal complexity in the last third of sustained isometric contractions of the biceps brachii was related to impaired peripheral factors, altered MU FR and recruitment strategies (synchronization was not measured).

Several authors investigated a possible correlation between indices of MU synchronization calculated from single fiber measurements and non-linear sEMG parameters, with controversial results. The studies of Del Santo and colleagues [1,42] reported a high correlation (R > 0.8) between the percentage of determinism and degree of synchronization during pharmacological interventions in several muscles of the upper and lower extremities. However, Dideriksen et al. [20] reported no association between the degree of synchronization and %DET in vastus medialis and abductor digiti minimi during a 2 min 5 % MVC contraction. Moreover, changes in other non-linear indices during isometric fatiguing contractions, such as multiscale entropy and correlation dimension were ascribed to MU synchronization, but authors provided no correlation analysis [43,44].

As already reported in literature, FD is sensitive to the presence of large active MUAPs that usually appear in the signal due to synchronization at high force levels during fatiguing contractions [5]. Nevertheless, a similar phenomenon happens also at low force levels, whenever larger MUs, with low firing frequency, are recruited according to the Henneman's size principle [45]. In addition, during simulated contractions, Mesin and colleagues [25] reported a dependency of FD on MU synchronization and changes in the FR patterns, even though a high force level (80 % MVC) was simulated, and MU synchronization was varied in the range 0-20 % (in step of 5 %). Our data seems to confirm the results obtained using synthetic signals, even though the correlations were not as high as would have been expected. Nevertheless, with experimental sEMG signals, it is much more complex and difficult to associate changes in non-linear parameters to physiological phenomena that may manifest during fatiguing contractions [46], such as synchronization of the MUs generating the action potentials, changes in the shape of action potentials, in the FR, or in the biochemical conditions and metabolism of the muscle fiber [34].

The key limitations are as follows. First, to estimate MU synchronization, the detection volume of iEMG and sEMG electrodes was different (i.e., few mm² vs. few cm², respectively). Second, since the protocol was complicated, a convenience sample of subjects was recruited, without calculation on the statistical power needed.

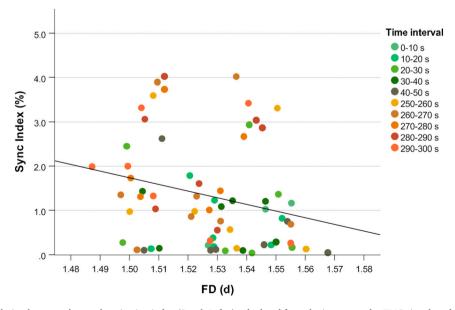


Fig. 4. Scatter plot of the relation between the synchronization index (Synch Index) calculated from the intramuscular EMG signal, and the fractal dimension (FD) of the surface EMG signal. Time interval refers to the initial and final 50 s of the isometric knee extension. $R^2 = 0.06$.

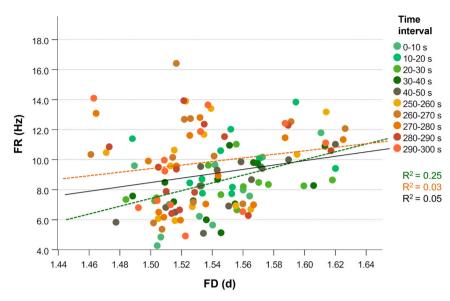


Fig. 5. Scatter plot of the relation between the firing rate (FR) calculated from the intramuscular EMG signal, and the fractal dimension (FD) of the surface EMG signal. Time interval refers to the initial and final 50 s of the isometric knee extension. The green and orange dashed lines represent the linear correlation between FR and FD during the initial and final 50 s, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Correlation results.

		Synch Index ^a		FR	
		r_s	p-Value	r_s	p-Value
FD	0–300 s 0–50 s (t1) 250–300 s (t2)	-0.30 -0.09 -0.18	0.03 0.65 0.31	0.25 0.52 0.20	0.004 <0.001 0.12

FD, Fractal dimension; Synch Index, synchronization index; FR, firing rate.

5. Conclusions

The results of this study indicate that the FD of the sEMG cannot be used as an exclusive index of MU synchronization during sustained fatiguing contractions, but rather as predominantly related to central factors. Indeed, at the beginning of contraction, or more in general when fatigue does not develop, FD is a generic index of signal complexity that merely remains correlated with FR, only. In addition, our results seem to indicate that MU synchronization occurs rather late in the contraction and that FD is an index of performance fatigability primarily at the end of a prolonged contraction, where signal complexity decreases. Because MU synchronization is linked to the CNS its study may provide an insight into functional neurophysiological aspects during voluntary tasks and pathological conditions. Therefore, further work should aim to develop a novel selective index of MU synchronization. However, FD as complexity parameter of the EMG signal, may be used in future studies to investigate the fractal properties of sEMG signals to characterize normal and pathological signals, to quantify the complexity of MU recruitment patterns, or to quantitatively assess muscle activity and fatigue.

CRediT authorship contribution statement

Matteo Beretta-Piccoli: Conceptualization, Methodology, Analysis and/or interpretation of data, Validation, Formal analysis, Investigation, Data Curation, Writing – Original draft, Writing – Review & Editing, Project administration. Corrado Cescon: Conceptualization, Methodology, Software, Analysis and/or interpretation of data, Validation, Formal analysis, Investigation, Data Curation, Writing – Original draft, Writing – Review & Editing. Ausilia Vistarini: Investigation, Resources, Data Curation, Project administration. Caterina Pisegna: Investigation, Resources. Beatrice Vannini: Investigation, Resources. Cristian

Zampella: Investigation, Resources, Data Curation. Luca Calanni: Investigation, Resources, Data Curation. Emiliano Soldini: Methodology, Validation, Formal analysis, Data Curation. Marco Barbero: Conceptualization, Methodology, Validation, Writing – Review & Editing, Supervision. Giuseppe D'Antona: Conceptualization, Methodology, Validation, Resources, Writing – Original Draft, Writing – Review & Editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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^a Analysis was conducted in nine subjects.

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