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### Nonlinear Analysis for Evaluation of Age-Related Muscle Performance Using Surface Electromyography

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#### 1. Introduction

Several electromyographic methods are currently used, but needle electromyography (nEMG) and surface electromyography (sEMG) are most often applied. To physiologically evaluate electromyographic wave patterns for the detection of abnormalities, the wave patterns obtained with nEMG or sEMG have been macroscopically examined, and subjectively judged by physicians.

- nEMG findings are used for the evaluation of whether a disorder is neurogenic or myogenic, and if it is both neurogenic and myogenic, they provide important information about whether it is acute, subacute, or chronic (KIMURA, 1989). However, the probe is a needle electrode, which is percutaneously inserted into muscular tissues.
- sEMG findings are used for various evaluations, such as the classification of trembling for the diagnosis of involuntary motion, the diagnosis or differential diagnosis of dystonia and spasm, and the identification of involuntary constrictor muscles (KIZUKA et al., 2006).
- sEMG is further used for the determination of the electric potential through a nerve conduction examination (evoked EMG). In evoked EMG, the electrostimulation of peripheral nerves is percutaneously performed (KIMURA, 1989).

The examination methods described, except for method B), are invasive and cause severe pain in patients. Generally, "smoothing" and "integration" refer to two ways of quantifying EMG energy over time; smoothing refers to continuously averaging out the peaks and valleys of a changing electrical signal. On the other hand, integration refers to measuring the area under a curve over a period of time. These methods are used to examine the relative degree of muscular contraction, and are also employed to provide a parameter for the evaluation of muscular training conditions (Aukee, 2002). However, the results obtained are affected by the location of the measuring electrodes and the shape and size of the probes. That is, EMG findings are macroscopically and subjectively evaluated, as described above, and no algorithm for the quantification of the degree of muscular abnormalities or recovery

has been established. In this study, we apply and discuss the measurement parameters that have been developed for evaluating the average rectified sEMG (ARS) data obtained from perineal muscles during biofeedback training (BFT) for the treatment of dysuria (Tries & Eisman, 1995) (Fig. 1), and we evaluated the effects of this training (SHIOZAWA et al., 2007). Kegel (1948; 1951) was the first to use BFT for the treatment of urinary incontinence (UI), and it was observed that if the pelvic floor muscles are hypotonic, bladder suspension surgery is less effective for treating stress-related incontinence. In order to improve the contractability of the pubococcygeus portion of the levator ani muscle, Kegel invented the pressure perineometer (KEGEL, 1951). In the U.S.A., at least 13 million community-living adults and more than 50% of all residents in nursing facilities suffer from UI (FANTL et al., 1996). The direct medical expenditure incurred for the care of these people is estimated to be >\$15 billion annually, in addition to the \$35.2 billion incurred annually for nursing home residents (PEEK et al., 1995). There is a consensus that in most cases, behavioral treatment modalities, including biofeedback, should be used before invasive modalities such as surgery.

A biofeedback instrument has three tasks (PEEK et al., 1995): i) To monitor (in some way) a physiological process of interest, ii) To measure (objectify) what is monitored, and iii) To present what is monitored or measured as meaningful information. The contributions of many previous researchers and practitioners can be cited as the forerunners of biofeedback.

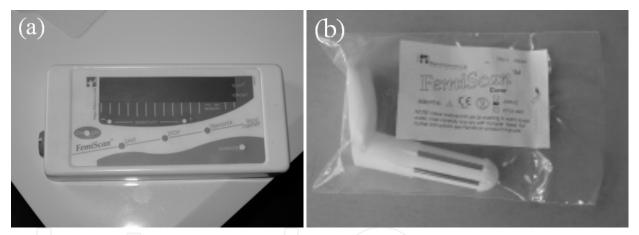


Fig. 1. A biofeedback system (FemiScan Co. Ltd., Finland). The main body (a), and a disposable electrode that can be inserted into perineal muscles (b).

Edmund Jacobsen commenced research at Harvard in 1908, and throughout the 1920s and 1930s worked to develop progressive muscle relaxation as an effective behavioral technique for the alleviation of neurotic tensions and many functional medical disorders (JACOBSEN, 1938). He used crude electromyographic equipment to monitor the levels of muscle tension in his patients during the course of treatment. The classification of and historical perspectives on biofeedback applications can be found in Gaarder and Montgomery (GAARDER and MONTGOMERY, 1981), Gatchel and Price (GATCHEL and PRICE, 1979), and Basmajian (BASMAJIAN, 1989).

Recently, the rapid atrophy of the muscles used for bending at the hip joint during walking (flexor muscles around the hip joint) with age has drawn attention. The flexor muscles around the hip joint consist of the femoral rectus and abdominal muscles. It has been indicated that a lack of these muscles is responsible for the falling of the elderly. In this

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study, we examined the ARS of the femoral rectus muscles performed during the BFT of the dominant leg, using the measurement parameters mentioned above, and evaluated changes with age.

#### 2. Sensor output signal evaluation system

The sensor output signal evaluation (SOSE) system was developed in 2006 (Shiozawa et al., 2006a). The SOSE system can evaluate the exponential curve-fitting in BFT.

#### 2.1 Participants

The subjects consisted of 31 healthy adults aged 20-73 years (mean, 44.3±19.9 years). All of the subjects were Japanese and lived in Nagoya and its environs. The following were the exclusion criteria for the subjects: subjects working in a night shift, subjects with a dependence on alcohol, subjects who consumed alcohol and caffeine-containing beverages after waking up and within two hours of eating a meal, subjects who may have had a previous history of bone, joint, or nerve problems, and special strength training exercises were not usually done. The subjects were not prescribed drugs for any disease.

#### 2.2 Design

The subject sat back on a four-legged stool, and electromyographic electrodes were applied at an interval of several centimeters to the center of the femoral rectus muscles in the dominant left or right leg (Fig.2). The subjects were instructed to kick a fixed belt by moving the bottom of the lower leg forward (kicking motion).

#### 2.2.1 Biofeedback training

Temporal data were obtained using sEMG, and they are expressed here as  $\{y(t)\}$ . Generally, sEMG data are recorded by a computer at 2 kHz. Here, the integral calculation was performed every 0.1 s using the following equation:

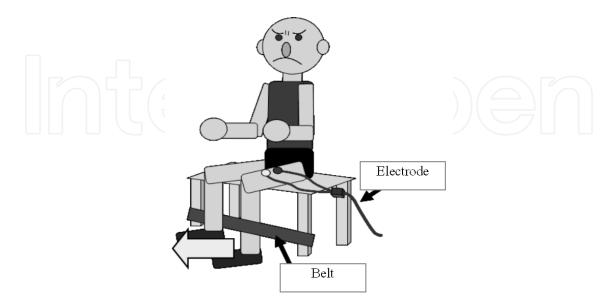
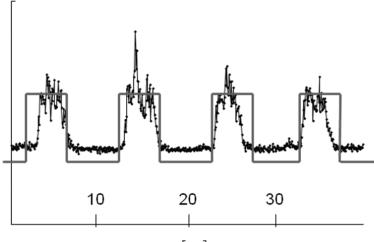


Fig. 2. Biofeedback training instruction signal. The BFT instruction signal produced by superimposing the ARS on the target instruction signal.

$$x(t) = \sum_{k=0}^{199} |y(t+0.0005k)|, \qquad (1)$$

and the ARS  $\{x(t)\}\$  was calculated in real time and outputted. The subject was told to observe the outputted wave patterns and the rectangular waves f(t) of a 10-s cycle superimposed on the same display (Fig. 3), and then perform intermittent continuous contractions of the femoral rectus muscles corresponding to the patterns (BFT).

The subjects performed BFT for 2 min. We ensured that the body sway was not affected by environmental conditions; using an air conditioner, we adjusted the *temperature* to 25 °C in the exercise room, which was large, quiet, and *bright*. All subjects were tested from 10 am to 5 pm in an exercise *room*. All subjects gave consent in writing after a sufficient explanation of this study.



time [sec]

Fig. 3. Biofeedback training instruction signal. BFT instruction signal produced by superimposing the ARS on the target instruction signal.

#### 2.2.2 Materials

A special electromyographic transformation box (AP-U027, TEAC Co.) was connected to a commercially available portable and versatile amplifier and recorder (Polymate AP1532, TEAC Co.), and electromyographic electrodes (bipolar) with preamplifiers were used.

#### 2.2.3 Procedure

First, the electromyographic wave patterns obtained over 5 seconds at the maximum effort of the kicking motion (maximum voluntary contraction (MVC) (CARLO and DELUCA, 1997) were integrated in real time using a computer, and the ARS data on the display were shown to the subject. Next, the threshold line at 75% of the mean ARS (mV) during the muscular contraction period was shown to the subject, who was requested to perform muscular training aiming at the threshold line for 1 min 20 s. In other words, BFT was performed at 75% of the MVC. During BFT, data were recorded in a notebook computer (AP Monitor, NoruPro) at a sampling rate of 2 kHz. The low frequency cut-off filters were used at 16 Hz, and an alternating current-eliminating filter was also used.

#### 2.3 Algorithm in the SOSE system

The initial 20 s of the sEMG data recorded over a total of 1 min 20 s were excluded, because the subjects may have required this time to adjust to the training. The sEMG data of the 6cycle rectangular waves that occurred over the remaining minutes of training (target value) f(t) and the ARS were analyzed in accordance with the Double-Wayland algorithm (TAKADA et al., 2006a) and our own mathematical algorithms of the sensor output signal evaluation system. Fig. 4 shows a revision of the flow chart in SHIOZAWA et al. (2006a). Taking a mean of the ARS (MARS) as a threshold H for determining continuous muscular contractions, the time sequences above the threshold H were regarded as continuous muscular contractions. Based on whether differences such as x(t) - x(t - 0.1) and (x(t + 0.1) - x(t))(x(t) - x(t - 0.1)) were positive or negative, a maximal series for the continuous muscular contractions was extracted as shown in Fig. 5.

- a. The value of the MARS during the muscular relaxation period (x<sup>a</sup>) and the measurement parameters in the other terms (SHIOZAWA et al., 2006b; SHIOZAWA et al., 2006c; TAKADA et al., 2006b) indicating the shape of the ARS were determined every cycle, and the ARS values obtained from the femoral rectus muscles were evaluated.
- b. Maximum amplitude (x<sup>b</sup>): This maximum value was examined and recorded.
- c. Duration of continuous muscular contraction (x<sup>c</sup>): The duration between the first and last maximal values in a cycle exceeding the MARS sEMG was measured (Fig. 5).
- d. The time constant of the exponential decay curve fit to the maximal points during the muscular contraction period in the BFT (x<sup>d</sup>): All maximal values between the first and last maximal values exceeding the MARS in a cycle were extracted as {x<sub>m</sub>(t)} and fit to the exponential decay curve  $\hat{x}_m(t) = \text{Cexp}[-x^d t]$ . On a semi-log graph, the time constant (x<sup>d</sup>) was estimated using the mean least square method, which minimized the sum of the squared residuals;

$$L = \sum_{t} \{ \log \hat{x}_m(t) - \log x_m(t) \}^2 .$$
<sup>(2)</sup>

In order to estimate the time constant (x<sup>d</sup>), the following simultaneous equation should be solved.

$$\frac{\partial L}{\partial \log C} = 0, \frac{\partial L}{\partial x^d} = 0$$
(3)

The numerical sequences of the 4 measurement parameters were determined at a repetition number of 6. The relationship between the age (z) of the subjects who had undergone sEMG and the value  $x^i(z)$  (i = a,b,c,d) estimated in the 5<sup>th</sup> cycle was statistically examined to evaluate correlations between each measurement parameter and age (Appendix).

#### 2.4 Practical study of the SOSE system

The numerical sequences of each of the measurement parameters at a repetition number of 6 were obtained by sEMG performed during BFT. The relationship between the age (z) of the subjects who had undergone sEMG and the value  $x^i(z)$  (i = a,b,c,d) was examined in 5<sup>th</sup> cycle. Fig. 6 shows the  $x^i(z)$  of all 50 subjects. The linear regression analysis using the least-square method, demonstrated that the coefficients by which age (z) was multiplied were 0.071, - 0.268, -0.006, and -0.010 for (a), (b), (c), and (d), respectively, and the parameters,

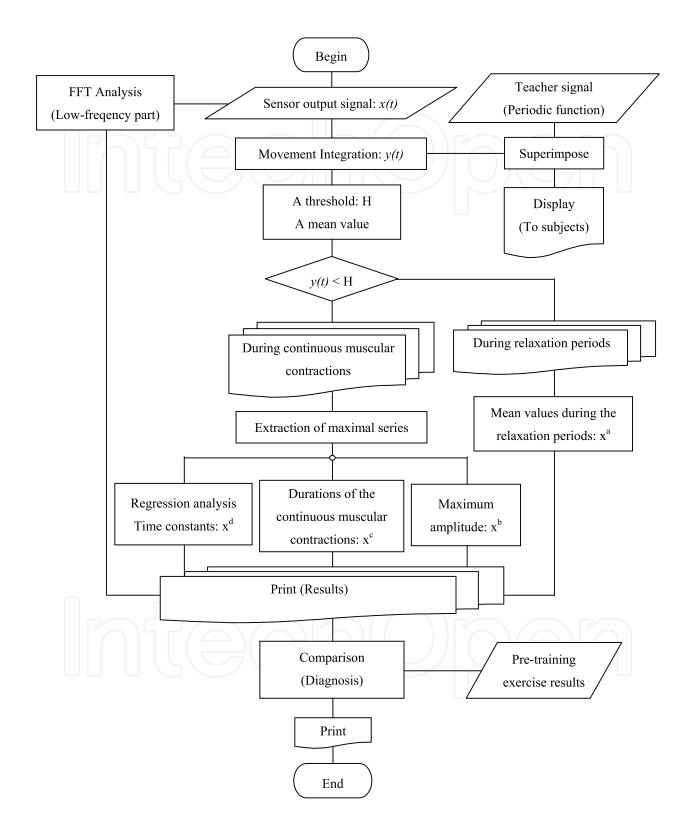


Fig. 4. A part of the flow chart of our mathematical algorithm of the sensor output signal evaluation system.

Nonlinear Analysis for Evaluation of Age-Related Muscle Performance Using Surface Electromyography

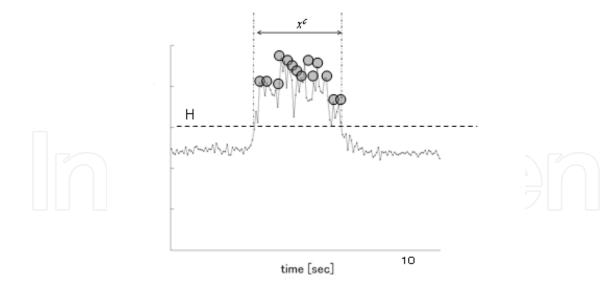


Fig. 5. A series of maximal values of the ARS during the muscular contraction periods in the BFT were extracted by our mathematical algorithm of the sensor output signal evaluation system.

except for (a), decreased with age. Since the linear regression coefficients varied with the measurement parameters, correlations between these parameters and age could not be judged using only the coefficients by which age (z) was multiplied. When using the t-test to evaluate the null hypothesis ( $\hat{b} = 0$ ) for the regression coefficient ( $\hat{b}$ ), the test values (A.3) were 1.105, 0.238, 1.621, and 3.245 for (a), (b), (c), and (d), respectively, and the only parameter exceeding t<sub>48</sub>(0.975) was the time constant of the exponential decay curve fit to the maximal points of the muscular contraction period in the BFT (x<sup>d</sup>).

We examined correlations between the parameters of the ARS and age. Regression equations (A.1) for each parameter (i = a,b,c,d) were determined, and the null hypothesis ( $\hat{b}$  = 0) for the regression coefficient ( $\hat{b}$ ) was examined using the t-test. Since the test value (A.3) was larger than the two-sided 5% point, t<sub>48</sub> (0.975), in the t distribution with a latitude of 48, the null hypothesis was rejected in the case of i = d. Therefore, the time constant of the exponential decay curve fit to the maximal points during the continuous muscular contraction period (x<sup>d</sup>) is significantly dependent on age (p<0.05).

#### 2.5 Problem

The analysis of sEMG data is generally performed by the fast Fourier transformation (FFT), which is a linear analytical method. A Fourier series expansion of the function f(t), showing target levels (instruction signals) for BFT, is possible, and the following expansion equation (MATSUMOTO and MIYAHARA, 1990) is useful for evaluating the differences between the ARS and the rectangular waves:

$$f(t) = 1 + \sum_{k=1}^{\infty} \frac{2}{k\pi} \{1 + (-1)^{k+1}\} \sin \frac{2\pi k}{T} t .$$
(4)

Higher-order terms (high-frequency components) are included in this expansion equation, but the coefficients are small due to the presence of the order in the denominator. Accordingly, the high-frequency power was considered to be theoretically irrelevant for the

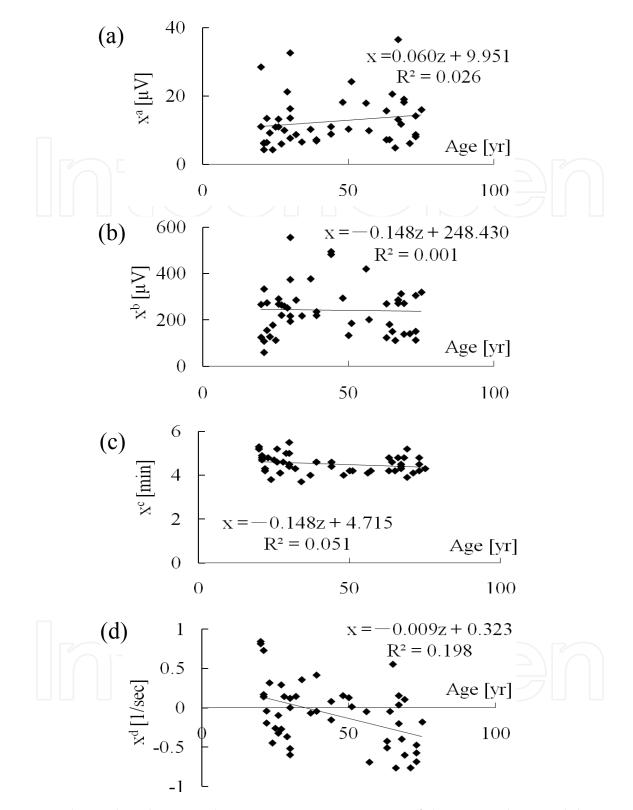


Fig. 6. Relationships between the measurement parameters of the ARS and age and their linear regressions. R<sup>2</sup> shows the coefficient of determination. MARS during the muscular relaxation period,  $x^a$  (a). Maximum amplitude,  $x^b$  (b). Duration of continuous muscular contraction,  $x^c$  (c). Time constant of the exponential curve fit to the maximal points of the continuous muscular contraction period,  $x^d$  (d).

evaluation shown in Eq. (4). Since muscular conditions always vary, signals should be regarded as non-steady (BEYER, 1987). Since a spectral estimation by the Fourier analysis is based on the assumption that the signals to be analyzed are steady and linear, this linear analysis of sEMG data is inappropriate.

Myopotentials are induced by changes in the firing patterns of nerve impulses. Several muscle fibers controlled by a motor nerve are collectively called a motor unit (MU), and several MUs can be excited by nerve impulses, causing an MU action potential. The MU action potential measured on the skin's surface is a superficial myopotential, and it is observed at a site spatially distant from the local region where the MU action potential waves are generated. In sEMG, a very large number of MU action potential waves are superimposed, and the activity states of whole muscles are observed by this method (YOSHIDA et al., 2004). Therefore, sEMG signals should be considered nonlinear, or more generally, sEMG shows a time series produced by stochastic processes. Recently, it has been recognized that sEMG data can be examined by nonlinear analytical methods, such as the recurrence plot and the Wayland algorithm (YOSHIDA et al., 2004; TAKADA et al, 2006c). However, the measurement parameters used in the present study, such as the time constant of the exponential decay curve fit to the maximal points of the continuous muscular contraction period (x<sup>d</sup>), are used as a linear analytical method for sEMG. In the next section, we discuss the reason why we have succeeded in findings a linear index showing a correlation with age.

#### 3. Solution & recommendations

The complexity of the bio-signal or the degree of visible determinism generating those signals can be measured by our Double-Wayland algorithm (TAKADA et al, 2006a), which is introduced in this section.

#### 3.1 Double-Wayland algorithm

The translation error is a statistical index that measures the smoothness of flow in an attractor generating a time series. In addition, randomness can be evaluated by the Double-Wayland algorithm by comparing the translation errors in the temporal differences of the time series (differenced time series) with the results of the Wayland algorithm in each embedding space (Wayland et al., 1993).

An attractor is reconstructed from a time series. The attractor is constructed by means of embedding the time series data proposed by Takens (1981) in the phase space. Embedding is a method that draws an orbit in phase space supposing a vector whose elements are the values for when the time elapses from *t* to  $\Delta t$ ,  $2\Delta t$ , ...,  $(N-1)\Delta t$  as a point in N dimensional phase space (embedding space). N and  $\Delta t$  are referred to as the embedding dimension and the sampling time, respectively. The delay coordinates {**x**(*t*)} can reconstruct a continuous trajectory without crossing into an embedding space that has a high dimension. If we only resample the time series at every delay time  $\tau$  when the auto-correlation coefficient  $\rho(\tau)$  is regarded as zero, components of the delay coordinates **x**(*t*) = (**x**(*t*), **x**(*t*+ $\tau$ ),..., **x**(*t*+(*N*-1) $\tau$ )) cannot linearly correlate with each other. In this study, the auto-correlation function  $\rho(t)$  was estimated from the time series data (Matsumoto et al., 2002) and regarded as zero when  $\rho(t)$  decreased below  $1/e \cong 0.37$  for the first time ( $t \ge 0$ ).

The Wayland algorithm assumes that the difference vectors  $\mathbf{v}(t) = \mathbf{x}(t+\tau) - \mathbf{x}(t)$  in the embedding space characterize the nonlinear variations of the trajectories and estimate the translation error in an *m*-dimensional embedding space (*m* = 1, 2, ..., 10). Here,  $\tau$  was estimated

at 73-76 times the sampling time. A linear correlation between adjacent vectors  $\mathbf{x}(t)$  and  $\mathbf{x}(t+\tau)$  is eliminated by resampling the time series with respect to each embedding delay  $\tau$ .

- i. A series of delay coordinate vectors  $\{\mathbf{x}(t)\}$  is embedded in each space.
- ii. M onset periods  $t_0$  are randomly selected.
- iii. The values of

$$E_{\text{trans}}(t_0) = \frac{1}{K+1} \sum_{i=0}^{K} \frac{|\mathbf{v}(t_i) - \overline{\mathbf{v}}|}{|\overline{\mathbf{v}}|}$$
(5)

are standardized by the average of the difference vectors at K+1 points  $\{\mathbf{x}(t_i)\}_{i=0}^{K}$ .

$$\overline{\mathbf{v}} = \frac{1}{K+1} \sum_{i=0}^{K} \mathbf{v}(t_i) \tag{6}$$

is obtained at every onset period, where the *K* points nearest to  $\mathbf{x}(t_0)$  are selected as  $\{\mathbf{x}(t_i)\}_{i=0}^{K}$ .

- iv. The median of the *M* values of Eq. (5) is extracted.
- v. *Q* medians are obtained by repeating the above steps. The translation error  $E_{trans}$  is estimated by the expectation value of these *Q* medians.
- The Double-Wayland algorithm includes the following additional steps.
- vi. Translation errors,  $E_{\text{trans}}'$ , are derived from temporal differences in the time series data (differenced time series) {**x**(*t*+ $\tau$ ) **x**(*t*)} by the Wayland algorithm outlined above.
- vii. If a differential equation system that included stochastic factors was the generator of the time series, the flow would not be smooth. In such a case, a significantly higher number of translation errors might be estimated in the last step than in step (v).

In this study, we set the conditions of the coefficients *M*, *K*, and *Q* to be 51, 3, and 10, respectively (Wayland et al., 1993).

If a time series is produced from a chaos process, the translation vectors point in almost the same direction unless the time implementation  $\tau$  is too large, since deterministic aspects remain in time development. The minimum translation error would be estimated in such an embedding space that has no false intersection along the orbit and best reflects the degree of freedom. Hereby, the optimum embedding dimension to capture the chaos process can be obtained.

The differenced time series produced in the stochastic process often reconstitutes an indifferentiable orbit in embedding space. This indicates that the translation error estimated from the differenced time series exceeds the translation error estimated from the time series data. Accordingly, we weighed the translation error estimated from the time series data against the error estimated from the differenced time series in m dimensional phase space.

In general, the threshold of the translation error for classifying the time series data as deterministic or stochastic is 0.5, which is half of the translation error resulting from a random walk (MATSUMOTO et al., 2002). The abovementioned  $E_{trans}$  is compared with the translation error ( $E_{trans}'$ ) estimated from the differenced time series.

#### 3.2 Non-linear analysis of the sEMG in the BFT

Using the Double-Wayland algorithm, translation errors were estimated from the sEMG measured during a continuous muscle contraction period and during BFT. We compared

the translation errors E<sub>trans</sub> and E<sub>trans</sub>' in each embedding space (Figs.7). Intermittent muscle the differenced sEMG's of the younger subjects. Etrans' would be less than Etrans if the degree of determinism involved in the generator were reduced. The form of a rectangular wave as a teacher signal might reduce the nonlinearity involved in the generator of sEMG for the young. Moreover, we employed the method of surrogate data to ascertain the cause of the correlation between age and a linear index for the ARS. According to the Fourier shuffle algorithm, 20 surrogate sequences were generated from each ARS during the 3 s continuous muscle contraction period and the 3 s of BFT shown in Fig. 7. Using the Wayland algorithm, translation errors were estimated from the surrogate sequences. The embedding delay was estimated as  $0.064 \pm 0.003$  s and  $0.290 \pm$ 0.135 s from the surrogate data of the ARS during the continuous muscle contraction period and BFT, respectively. A significant difference was observed between the translation errors, Etrans, estimated during the continuous muscle contraction period and the BFT (p < 0.01). The E<sub>trans</sub> calculated during the continuous muscle contraction period was greater than that taken after this period. BFT could enhance the linearity in the generator of sEMG.

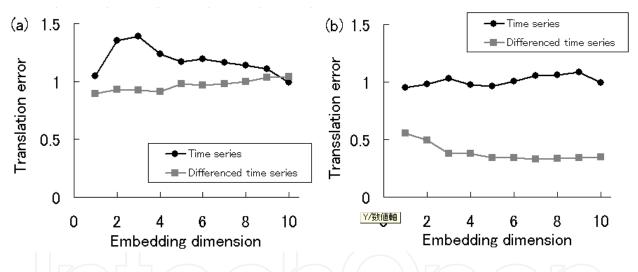


Fig. 7. Results of the calculations involved in sEMG using the Double-Wayland algorithm. Translation errors, E<sub>trans</sub> and E<sub>trans</sub>', were estimated from sEMG data measured during a continuous muscle contraction period for 3 s (a) and during 3 s of BFT (b) (TAKADA et al., 2010).

#### 3.3 Stability of the sEMG in the BFT

Since there were differences in not only the unit of measurement but also the numerical order between the parameters, they were normalized using the intermediate values  $\bar{x}^i$  for each cycle, and the reproducibility (stability) of the measurements was evaluated using the standard deviation  $\sigma[x^i/\bar{x}^i]$ . The normalized value is 1 when the measurement is equal to the intermediate value. When the reproducibility (stability) of repeated measurements is high, the deviations from this value are small, and the standard deviation is close to 0.

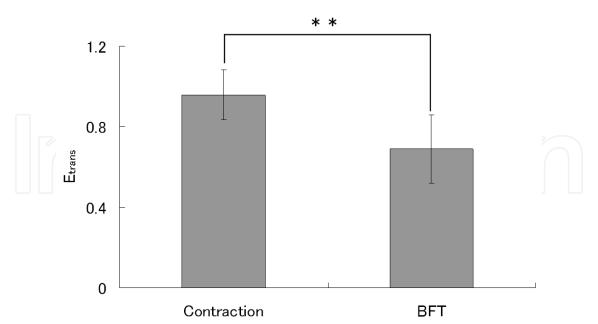


Fig. 8. Result of the surrogate data analysis. In 10 dimensional embedding space, translation errors were estimated from the surrogate data of the ARS during the continuous muscle contraction period and the BFT.

The intermediate values of the parameters and the standard deviations ( $\sigma$ ) of normalized measurements were determined for each subject, and the medians of  $\sigma$  in the age groups are compared in Table 1. The duration of continuous muscular contraction (x<sup>c</sup>) alone showed  $\sigma$  < 0.1 for any age group.

Age group	Ν	Mean during relaxation	Maximal amplitude	Duration of continuous muscular contraction	Time constant
≤25	8	0.24	0.13	0.06	1.51
≤45	9	0.21	0.14	0.08	0.55
≤65	6	-0.33	0.14	0.08	1.84
65 <	8	0.07	0.06	0.06	0.89

*N* expresses the number of subjects in each age group. Table 1. Standard deviations of normalized indices  $x/\overline{x}$  <sup>8</sup>

#### 4. Future research directions

The decline in translation errors,  $E_{trans}'$ , was not always seen in the middle-aged and the elderly. Poor muscular control might enhance the instability of the temporal variations involved in the ARS, which should be maintained as a constant during a muscular contraction in BFT. The relationship between age and the decline in the translation errors,  $E_{trans}'$ , should be investigated in future work.

We will employ a time series analysis, such as a surrogate method, to ascertain the cause of the correlation between age and a linear index of ARS.

#### 5. Conclusion

Recently, there has been an increasing focus on the rapid reduction of the muscles that are required for bending the hip joint during walking with age. Atrophy of the flexor muscles has been implicated in falling in the elderly. In this study, we examined the ARS of the femoral rectus muscles during BFT of the dominant leg. To this end, we developed parameters for the measurement of the shapes in the ARS, and evaluated the changes in these parameters as the muscles age. A statistical analysis indicated that it was necessary to include the time constant of the exponential decay curve fit to the maximal points during prolonged muscular contraction to evaluate changes with age using the ARS during BFT. A reduction in the function of muscular control due to aging can be detected by performing sEMG during BFT using this time constant. Using our Double-Wayland algorithm, we have also confirmed the stationarity of the ARS during the muscle contraction period.

#### 6. Appendix

The relationship between each of the above measurement parameters and age was examined.  $x_j^i(z_j)$  of subject j ( $j = 1, 2, \dots, 50$ ) was plotted, and a linear regression analysis of these 50 points was performed by the least-square method (MATSUMOTO and MIYAHARA, 1990). The regression equation of each measurement parameter was determined.

$$\mathbf{x}^{\mathbf{i}} = \hat{a} + \hat{b} \mathbf{z} \tag{A.1}$$

s.t. 
$$\hat{a} = \frac{1}{50} \left( \sum_{j=1}^{50} x_j^i - \hat{b} \sum_{j=1}^{50} z_j \right),$$
 (A.2.1)

$$\hat{b} = \frac{1}{S_{zz}} \sum_{j=1}^{50} x_j^i \left( z_j - \frac{1}{50} \sum_{j=1}^{50} z_j \right). \quad (i = a, b, c, d)$$
(A.2.2)

Here,  $S_{zz}$  denotes a variance of age. The dependence of each measurement parameter on age was statistically evaluated by a two-sided t-test with the null hypothesis that the regression coefficient  $\hat{b} = 0$ .

$$\left|\hat{b} - 0\right| / \sqrt{S_E / 48S_{zz}} \tag{A.3}$$

If the above value is larger than  $t_{48}(1-\alpha/2)$ , the null hypothesis is rejected, and the measurement parameter is considered to be correlated with age (SHIMIZU and TAKADA, 2001). Here,  $S_E$  denotes the residual sum of squares by the least-square method, and  $t_{48}(1-\alpha/2)$  represents the t distribution at a probability of  $1-\alpha/2$  and a latitude of 48. In this study, since the significance level ( $\alpha$ ) was defined as 0.05,  $t_{48}(1-\alpha/2)$  was approximately 2.010.

#### 7. Acknowledgment

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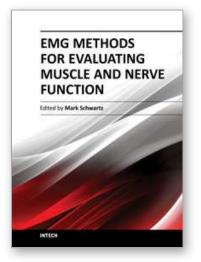
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