








Normalization of the electromyographic signals of masticatory muscles during non-habitual chewing activity

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There is no consensus on the most appropriate method for normalizing electromyography (EMG) signals from masticatory muscles during isotonic activity. **Aim:** To analyze the best method for data processing of the EMG signal of the masticatory muscles during isotonic activity (non-habitual chewing), comparing raw data and different types of normalization. **Methods:** This is a cross-sectional study. Women aged between 18 and 45 years were selected. Anthropometric data were collected (age, height, body mass index – BMI, masticatory preference) as well as EMG signal (root mean square – RMS) data for the anterior temporal and masseter bilaterally, and for the suprahyoid muscles, during isotonic (non-habitual chewing) and isometric tasks. EMG data were processed offline using Matlab® Software. The normalization of the EMG signal was carried out using the 2nd masticatory cycle, chosen at random, of the 20 cycles collected, the maximum RMS value, and the maximum voluntary contraction (MVC). To analyze the best method of data processing for the isotonic data, the coefficient of variation (CV) was calculated. Descriptive data analysis was adopted, using the mean and standard deviation. ANOVA with repeated measures was used to detect significant differences between the methods of normalization. Statistical significance was set at 5% ($\alpha < 0.05$). **Results:** The final sample of this research was composed of 86 women. The volunteers presented an average age of 27.83 ± 7.71 years and a mean BMI of 22.85 ± 1.91 Kg/m². Regarding masticatory preference, 73.25% reported the right side, and 26.75% the left side. Considering the comparison between the methods, the

%CV measure of the 2nd cycle showed the lowest variation coefficient during biting for all the muscles from the raw data, RMS Max, and MVC ($p=0.001$, $p=0.003$, and $p=0.001$ respectively). **Conclusion:** In conclusion, for non-habitual chewing activity, the results of this study recommend data processing using normalization with the second cycle during chewing.

Keywords: Electromyography. Masticatory muscle. Mastication. Muscles.

Introduction

With the increase in the use of surface electromyography (EMG) in clinical and academic practice, it is extremely important to develop protocols to ensure the correct interpretation of data, such as the normalization of the EMG signal¹. Adherence to standardized methods allows the acquisition of reliable and valid data that, in turn, facilitates the interpretation and correct comparisons between the results obtained². The current literature reports the need to standardize the EMG signal normalization procedure³.

EMG normalization is a mathematical procedure, whereby the absolute EMG data is divided by a reference value⁴. Anatomical and physiological factors can significantly influence the amplitude of the electromyographic signal, leading to high variation and heterogeneity in the signal for comparison. To truly compare the EMG signal between individuals, muscles, and/or days, normalization is necessary⁵⁻⁷.

A recent systematic review found that only 50% of studies with electromyography (that evaluated the isotonic condition) of the masticatory muscles described the method used to normalize the data, and there is no standardization⁸. It is worth mentioning that in the research carried out by the authors, studies were found that evaluated normalization for isometric conditions, however, no studies were found that evaluated normalization for isotonic conditions taking into account the masseter, temporal, and suprahyoid muscles. There are many ways that the literature shows to normalize the EMG data. The maximum isometric contraction (MVC) is a common method of normalization, however, individuals with control-limiting conditions or muscle weakness are unable to efficiently participate in this method⁹. The maximum RMS and dynamic tasks (ie. second cycle) are also usual to normalize this data. But there is no consensus in the literature regarding this topic in the masticatory muscles during isotonic activity.

The current study is justified due to the heterogeneity of the studies that use EMG for masticatory muscles and, in addition, to analyze the different forms of normalization and propose the format with the least data variability.

The importance of using normalization of the EMG signal that reduces signal variability while preserving its characteristics is known⁷, therefore, it is important to define the best way to process the data. Within the literature search carried out by the authors, this is the first study to compare raw data and methods of normalization during isotonic activity. Therefore, the current study aimed to analyze the best method of data

processing of the EMG signal of the masticatory muscles during isotonic activity (non-habitual chewing) comparing raw data and different types of normalization.

Methods

Study design

This is a cross-sectional study, approved by the Research Ethics Committee of the Local University, protocol n°. 25/2015.

Subjects

A sample size calculation was performed, based on a pilot study, composed of 20 volunteers. The outcome used was surface EMG, the main outcome of the present study. Considering the evaluated muscles, the mean normalized RMS value of the anterior temporal muscle during the biting phase was considered because it has a lower standard deviation value ($86.25 \pm 7.42\%$). According to the variables, an effect size of 0.34 was found. After setting power of 95% and a 5% alpha, an n of 82 volunteers was determined. The sample size calculation was performed using GPower® software, version 3.1.9.2.

The volunteers were recruited in a Surgery Sector of a School of Dentistry in a city in the interior of São Paulo state, Brazil. The volunteers were recruited through an advertisement at the university between January and December of 2017. All volunteers signed a statement of informed consent.

Inclusion and Exclusion Criteria

Healthy women aged between 18 and 45 years, with a Body Mass Index (BMI) $< 25 \text{ kg/m}^2$ were selected for the study. Volunteers who were toothless, wear dental prosthesis, and/or present osteoarthritis of the temporomandibular joint were excluded. The sample consisted only of women because this study intended to perform analysis with electromyography, so the inclusion of both sexes in the same comparison is not indicated.

Materials

Electromyography

The EMG 830C signal acquisition module (EMG System do Brasil, São José dos Campos, Brazil) was used for reading the sEMG signals, with an impedance of $> 10 \text{ M}\Omega$, analog/digital converter, 16-bit resolution, sampling frequency of 2000 Hz, and fourth-order Butterworth filter (high-pass filter set at 20 Hz and low-pass filter set at 1000 Hz).

Five differential surface electrodes were used, (self-adhesive Ag/AgCl electrodes had a conductive gel), with a fixed inter-electrode distance of 10 mm, gain of 20x, common rejection mode greater than 130 dB, input impedance of 10 G Ω , and signal/noise ratio of less than 3 μV RMS.

The skin was cleaned with 70% alcohol before the placement of the electrodes, positioned following the criteria proposed by Cram¹⁰. In addition, a reference electrode (30 × 40 mm) consisting of a metal plate was positioned on the manubrium of the sternum.

On the anterior temporalis muscles, the electrodes were positioned vertically, 3cm along the zygomatic arch, just lateral to the eyebrow. On the masseter muscles, the electrodes were positioned parallel to the muscular fibers, between the cheekbone and the corner of the jaw, with the upper pole of the electrode at the intersection between the tragus-labial commissure and the evocation-gonion lines. On the suprahyoid muscles, one electrode was fixed on the midline under the chin, running in the anterior-to-posterior direction, over the muscle mass felt in the submandibular region^{10,11} (Figure 1).

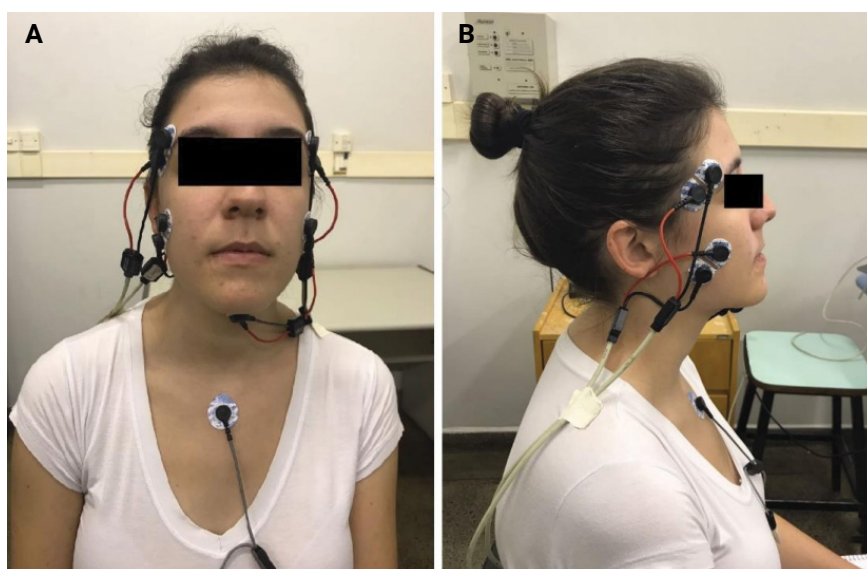


Figure 1. Positioning of the different bipolar electrodes on the masseter, anterior temporal, and suprahyoid muscles, and electrode monopolar reference: a) anterior view and b) profile.

Procedures

Anthropometric data were collected (age, height, body mass). To collect the EMG signal, all volunteers were asked to sit on a chair with their feet flat on the floor, hands on the lower limbs (knee and hip 90°), feet and chair on a rubber mat, and head parallel to the ground, concerning the Frankfurt plane. For the data collection, isotonic and isometric tasks were performed as described below (Figure 2):

Non-habitual chewing

A sheet of Parafilm M® (American National Can TM, Chicago, IL, USA) was used, folded three times in length and then in the middle in width. The volunteers were

required to position the Parafilm M® on the occlusal surfaces of the first and second upper and lower molars bilaterally during collection to protect the teeth^{11,12}. Thus, the volunteers were familiarized with the task. Two chewing repetitions were requested for 20 seconds each. The chewing was required to be carried out according to the rhythm of an MA-30 digital metronome KORG brand (New Market, USA), set at 60 beats per minute. For each repetition of chewing, the volunteers performed 20 full mouth biting/opening cycles, one cycle per second according to the pre-determined rhythm of the metronome. The volunteers were previously trained to perform the task. One cycle was considered as one bite and open.

Isometric procedure

The volunteers were asked to perform a bilateral molar bite on a dynamometer, with the maximum voluntary contraction (MVC). They were asked to bite with maximum strength, even if they felt pain in the temporomandibular joint. The MVC was collected for 5 seconds, and this evaluation was performed twice.

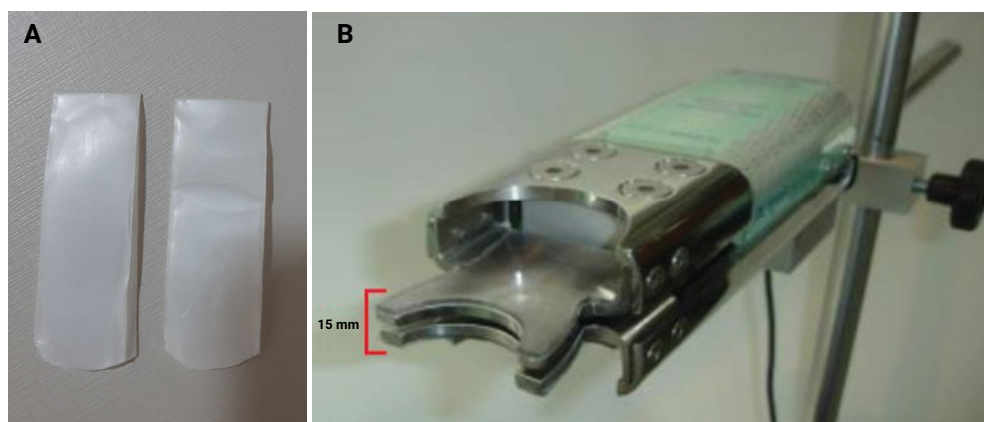


Figure 2. A) Sheet of Parafilm M®; B) Bite dynamometer.

Data Processing

EMG data were processed offline using Matlab® Software 8.5.0.1976.13 (R2015a, MathWorks Inc., Natick, Massachusetts, USA). For the processing of the signal, the cycles of activity were used. One phase of clenching the teeth (a contraction of the mandibular elevating muscles, denominated the biting phase) and mouth opening (a contraction of the depressor muscles of the mandible, denominated the mouth opening phase) was defined as the masticatory cycle.

Six central masticatory cycles of each collection in the EMG signal were considered to avoid interferences that could have occurred at the beginning and end of the collection, as well as to guarantee the standardization of the signal analyzed. In this way, the average of the sum of all the RMS values of the six cycles performed was used. It is important to note that all muscles started and ended the contraction of the biting

phase simultaneously, and there were no changes in the action potential of the motor units in the agonist muscles of the biting phase.

The six central masticatory cycles of each collection were manually selected through Matlab® Software. Three cuts were performed for each of the six cycles selected in the analysis for each attempt by the volunteers. The first cut was performed at the beginning of the biting phase, the second cut at the end of the biting phase (which also represented the beginning of the mouth opening phase), and the third cut at the end of the mouth opening phase (which in turn represented the beginning of the next cycle - biting phase).

For the statistical analysis, the mean of the six cycles of each repetition was calculated, to obtain the average of the electrical activity of each muscle (anterior temporal and masseter bilaterally and suprahyoid) in the biting phase and the average of each muscle in the mouth opening phase.

For the EMG signal processing and maximum bite force, a 4th order digital Butterworth filter was applied to the EMG signal, with zero phase delay (high pass of 10 Hz, low pass of 400 Hz). The first and second EMG signals were always eliminated to avoid interferences that occurred at the beginning and end of each collection. The EMG indices were processed in the amplitude domain to determine the root mean square (RMS) values, through the evaluation of the magnitude of the electrical activity of the masticatory muscles during the MVC. From this process the result was the raw data.

Subsequently, three different methods were performed to normalize the electromyographic signal, as follows: the second masticatory cycle of the 20 cycles collected, which was chosen at random; the maximum RMS value; and the MVC. The normalization is usually calculated by dividing the raw electromyographic data by a reference value, in this case, the normalization of the EMG signal during the biting and mouth opening phases of the muscles using each normalization value (second masticatory cycle, maximum RMS, and medium RMS) was performed using the following formulas⁴:

$$\left(\frac{\text{mean RMS of the six cycles in the bite phase}}{\text{normalization value}} \right) \times 100 ; \left(\frac{\text{average RMS of the six cycles in the mouth phase}}{\text{normalization value}} \right) \times 100$$

Statistical Analysis

To analyze the best normalization method for the isotonic data the coefficient of variation (CV) was calculated. The CV is a relative measure of variability that indicates the size of a standard deviation from its mean. It is a standardized, unitless measure that enables comparison of the variability between disparate groups and characteristics¹³. The CV was calculated for the raw signal, and for the three types of normalization (i.e., second masticatory cycle, maximum RMS, and MVC), to verify which normalization method presented the smallest variation.

The CV measures the relationship between the standard deviation and the mean. Thus, lower values indicate a more homogeneous data set. The values are expressed in percentages. A CV is considered low (indicating a reasonably homogeneous data-

set) when it is less than or equal to 25%. The formula used to calculate the coefficient of variation was described by Reed et al. (2017), as follows:

$$CV = \frac{\sigma}{\bar{X}} \cdot 100$$

The descriptive data are expressed in mean and standard deviation. To compare the means of each four methods of data processing and detect significant differences with a fewer type I errors, Analysis of Variance (ANOVA) with repeated measures was used. Statistical significance was set at 5% ($\alpha < 0.05$). Data analysis was performed using SPSS software, version 13.0.

Results

The final sample of this research was composed of 86 women, according to the flow-chart shown in Figure 3.

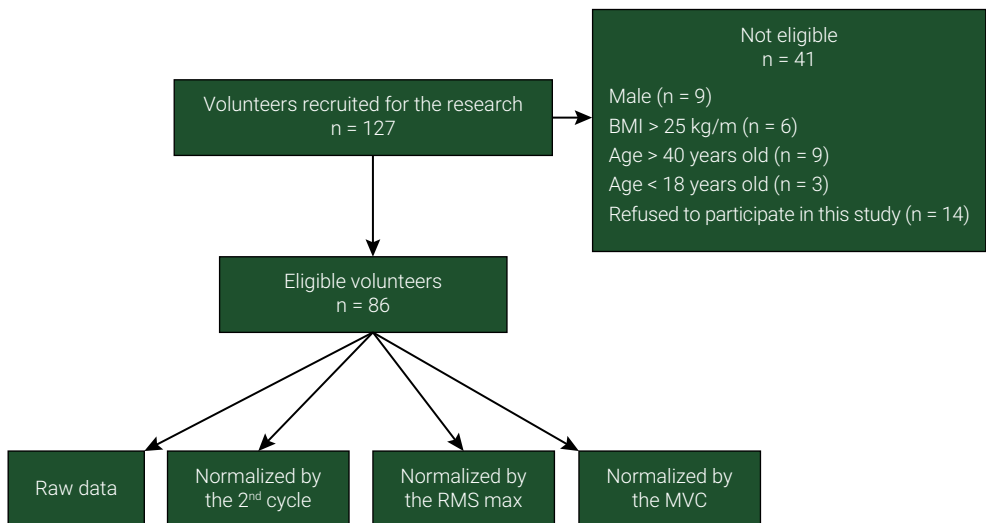


Figure 3. Flowchart of the recruitment of volunteers.

The volunteers presented an average age of 27.83 ± 7.71 years and a mean BMI of 22.85 ± 1.91 Kg/m². Regarding masticatory preference, 73.25% (63 volunteers) reported preferring the right side, and 26.75% (23 volunteers) preferred the left side.

Table 1 presents the mean, standard deviation, and coefficient of variation (CV) values for raw data and data normalized by the second cycle, maximum RMS, and MVC. This value shows what is expected in the literature. In the mouth opening phase, the suprahyoid muscles are in contraction therefore, their muscle activity is greater than other muscles (temporalis and masseter) which are at rest. In the biting phase is the opposite, the suprahyoid muscles are at rest and the temporalis and masseter are in contraction because they are mandibular elevators, so, they have a higher muscle activity.

The CV values are expressed in percentages for the masticatory muscles (temporalis and masseter bilaterally), for the mouth opening and biting phases. A CV with values lower than 25 % is considered low which means that it indicates a reasonably homogenous dataset. Therefore, normalizing by 2nd Cycle value would be ideal

Table 1. Mean, standard deviation (SD), and %CV values for raw data, data normalized by the second cycle, maximum RMS, and MVC, for the phases of mouth opening and biting (n=86).

	Muscle	Raw data		2 nd Cycle		RMS Max		MVC	
		Mean±SD	%CV	Mean±SD	%CV	Mean±SD	%CV	Mean±SD	%CV
Mouth Opening	LT	7.24±4.12	56.91	94.77±31.30	33.03	2.05±1.49	72.68	6.28±4.83	76.91
	RT	8.25±5.42	65.70	94.60±34.22	36.17	1.95±1.28	65.64	6.99±6.93	99.14
	LM	9.13±6.96	76.23	96.97±33.37	34.41	1.77±1.31	74.01	5.64±5.46	96.81
	RM	8.54±5.24	61.36	97.98±40.01	40.83	1.68±1.31	77.98	5.25±5.63	107.24
	SH	28.22±34.62	122.68	86.52±18.46	21.34*	23.49±10.70	45.55	84.25±60.22	71.48
Biting	LT	143.09±61.51	42.99	90.37±10.47	11.59*	35.84±6.47	18.05*	114.84±57.55	50.11
	RT	166.86±77.23	46.28	90.83±11.13	12.25*	36.53±6.04	16.53*	125.99±75.57	59.98
	LM	207.99±114.33	54.97	88.34±12.88	14.58*	34.73±6.02	17.33*	114.89±83.29	72.50
	RM	207.63±89.23	42.98	88.52±13.53	15.28*	34.55±6.15	17.80*	105.96±59.77	56.41
	SH	26.96±29.87	110.79	86.09±20.59	23.92*	20.77±8.45	40.68	85.93±81.69	95.07

RMS=Root Mean Square; MVC=Maximum Voluntary Contraction; %CV=% Coefficient of variation; LT=Left Temporalis; LM=Left Masseter; RT=Right Temporalis; RM=Right Masseter; SH=Suprahyoid; *CV low (indicating a reasonably homogeneous dataset).

Regarding the comparison between the methods, the measure %CV of the 2nd cycle showed the lowest variation coefficient during the biting phase for all the muscles from the raw data, RMS Max, and MVC (p=0.001, p=0.003, and p=0.001 respectively).

Discussion

The current study aimed to analyze the best method of data processing of the EMG signal of the masticatory muscles during isotonic activity (non-habitual chewing) comparing raw data and different types of normalization. Normalization by the second masticatory cycle showed the lowest variation coefficient during biting for all the muscles, followed by the RMS maximum for the mandibular lift muscles (anterior temporalis and master bilateral). However, during mouth opening, values lower than 25% were found only for the second cycle for the suprahyoideos, which is the agonist of the task.

EMG studies with dynamic activities such as walking and cycling have been widely studied in the literature and provide a lot of information about normalization¹⁴⁻¹⁷. The result of the present study corroborates the study of Albertus-Kajee et al.¹⁴, which indicates that the use of static isometric methods is not appropriate for the normalization of EMG signals in dynamic tasks.

An isometric maximal voluntary contraction (MVC) is mostly used for EMG normalization, a procedure described in the scientific literature to compare muscle activity among different muscles and subjects. However, the use of MVC presents certain limitations¹⁷.

In the second cycle, muscle activation is more stable, so variability is lower. Normalization by MVC demonstrates very different contractions in terms of action and pattern of MVC biomechanics and motor control.

The normalization procedure of the electromyographic signal, when performed from dynamic contractions, can be influenced by extrinsic factors of data collection, such as electrode displacements during movements. However, in addition to the current study, others that aim to verify the reproducibility of measurements of normalization procedures from the values in dynamic and isometric contractions indicated the use of normalization by the mean and peak of the EMG signal during dynamic activity, due to the lowest coefficients of variation found¹⁸.

Regarding contributions to clinical practice and research, for clinicians, publications that include isotonic evaluations are an advantage, since this enables understanding of how muscles behave in functional tasks and not only during isometric tasks. Considering research, this study demonstrates standardized methods to process and normalize the data, which also facilitates future comparisons among studies and provides more reliable results.

As strengths of the study, this is a pioneer study, since in the search carried out by the authors, no studies were found that address normalization during mastication of the temporal, masseter, and suprahyoid masticatory muscles. In addition, assessment of the suprahyoid muscle is rare. The present study is also relevant because it evaluates a more functional activity than an isometric task. The method used to analyze the cycle is also a differential of the study due to its complexity.

As a limitation, it can be pointed out that the collection was not carried out more than once, to test the reliability of the measurements. It is suggested that future studies carry out analyses to verify which normalization has a lower CV of the masticatory muscles at rest and in isometry conditions.

In conclusion, for non-habitual chewing activity, the results of this study recommend data processing using normalization with the second cycle during chewing.

Conflicts of interest

The authors have no personal or financial conflicts of interest related to the present work.

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Author Contribution

Elisa Bizetti Pelai: Conceptualization, Methodology, Data curation, Data analysis, Writing- Reviewing and Editing; **Ester Moreira de Castro Carletti:** Conceptualization, Methodology, Data curation, Data analysis, Writing; **Fabiana Foltran Mescollotto:** Conceptualization, Writing- Reviewing and Editing; **Paulo Fernandes Pires:** Conceptualization, Methodology, Data curation, Data analysis, Writing; **Fausto Berzin:** Conceptualization, Methodology; **Marcio de Moraes:** Conceptualization, Methodology, Writing- Reviewing and Editing **Delaine Rodrigues Bigaton:** Conceptualization, Methodology, Writing- Reviewing and Editing.

All authors actively participated in the manuscript's findings, and have revised and approved the final version of the manuscript.

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