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Standardized mastication increases the coordination in masticatory activity in women with chronic temporomandibular joint disorders: a case control study.

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ABSTRACT

Background: The effects of jaw movement pattern on masticatory activity during chewing remains unclear in chronic temporomandibular joint disorders individuals. Objective: to assess the effect of habitual and non-habitual mastication patterns based upon the activation of the masseter and temporalis muscles in individuals with or without temporomandibular joint disorder (TMJD). Methods: Fifty-four participants (age: 18–44 years) were divided into two groups: the TMJD (n=27) and control (n=27) groups. TMJD was identified using the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD). Electromyographic activity of the masticatory muscles was measured during 2 tasks: habitual mastication with parafilm (HM) and non-habitual mastication with parafilm (NHM). MATLAB software was used to process electromyography (EMG) signals. The root mean square, symmetry index (SI%), anteroposterior coefficient (APC%) and torque coefficient (TC%) were determined from the processed EMG signal. Results: Reduced right masseter activation was observed for the TMJD group (p<0.05) during jaw agonist phase. During the jaw agonist phase, all muscles presented with more activation during NHM. Symmetry of temporalis (ST%) and APC% were the lowest for HM. TC% was increased for HM. Conclusion: Habitual and non-habitual mastication differ in masticatory activity during jaw agonist and antagonist phase and TMJD individuals presented a different way to recruit muscles under these circumstances. Non-habitual mastication has a more coordinating and stable motor pattern in masticatory activity and has less variability than habitual mastication to assess masticatory activity.

Keywords: Mastication; Electromyography; Temporomandibular Joint Disorder; Masticatory Muscles; Motor Activity.

INTRODUCTION

Temporomandibular joint disorder (TMJD) is a functional or pathologic condition with higher prevalence in women than in men⁽¹⁾. It involves clinical manifestation of pain in the masticatory muscles, temporomandibular joints (TMJs), and other tissues⁽²⁾ that affect mastication^(3, 4), swallow⁽⁵⁾ and other orofacial functions. In TMJD, orofacial pain affects masticatory motor control⁽⁶⁻¹⁰⁾, and there is a reorganization in muscle activity⁽⁵⁾ with higher asymmetry in recruiting masticatory muscles during chewing resulting in worse functional performance compared to healthy individuals⁽³⁾, impaired muscle efficiency in terms of force contraction⁽⁸⁾, and altered mandibular function.

Considering that the activation of the masticatory muscles is altered in subjects with TMJD (3-12), i.e., increased masticatory muscle at rest ^(7, 10, 11), reduced or asymmetric activation during clenching and chewing ^(3, 4, 9-14), it is vital to investigate the behaviour of masticatory muscles during oral motor tasks to understand how this dysfunction influences muscle recruitment. However, the procedures required to evaluate masticatory function change across studies (3, 4, 9-11, 13-17), once the methodology applied is based on the expertise of researchers in their respective field. Several protocols have been proposed and used to investigate the neuromuscular balance of masticatory muscles during chewing.

What and how to chew for masticatory pattern evaluation change across studies. Studies have evaluated masticatory activity in the intercuspal position on participants' occlusal surfaces, using mostly cotton (3-5, 13), parafilm (9-11, 14, 16), chewing gum^(3, 4, 13, 17) and food^(15, 17). In relation to masticatory pattern, muscle activation has been measured during habitual (participants' pace)^(3, 4, 13, 15, 17, 18) and non-habitual masticatory pattern (controlled pace)^(10, 11, 14, 16). However, the effects of

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jaw movement in muscle activity in individuals with TMJD remain unclear. Also, mastication has been well-known to occur either unilateral or bilateral, and there is a preference for chewing side ^(19, 20) once chewing has intrinsic characteristics.

Given the changes occurring in the masticatory muscles in individuals with TMJD, the higher prevalence in women, and the approach used for masticatory evaluation, it is not known how the movement pattern affects masticatory muscle activation. Understanding how movement patterns affect the neuromuscular balance during masticatory muscles activation is fundamental to improve evaluation protocols and therapeutic intervention for this population.

In this study, we sought to assess the effect of habitual and non-habitual mastication patterns based upon the activation of the masseter and temporalis muscles in individuals with or without TMJD. We hypothesized that mastication pattern will affect masticatory activation

METHODS

Participants

The numbers of participants were calculated based on the study from Ries et al.¹¹⁾. Considering $\alpha = 0.05$, $\beta = 0.78$, and a test power of 80%, a minimum of 27 individuals in each group was required for TMJD and control group (asymptomatic group). Groups were paired by age, weight and height.

Inclusion criteria for the TMJD group were participants with chronic TMJD pain (>6 months). Exclusion criteria were participants who presented with Angle's levels II and III; had dental caries and missing molar tooth; experienced teeth pain; currently used dental retainers, occlusal appliance in the last six months, and analgesics; previous or current tumors or traumas in the head and neck region. The Angle's classification is based on where the buccal groove of the mandibular first molar contacts the mesiobuccal cusp of the maxillary first molar: on the cusp (Class I, neutroclusion, or normal occlusion), distal to the cusp by at least the width of a premolar (Class II, distocclusion), or mesial to the cusp (Class III, mesiocclusion). The inclusion of angle class I occlusion was done to have a uniform morphological aspect of dental occlusion. The study protocol was approved by the local research ethics board and registered under the number 758.038/2014 and all participants gave written informed consent to participate.

Clinical examination

One trained individual performed the clinical examination of all participants in accordance to the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD)⁽²⁾ to verify the presence and absence of TMJD. The participants of this case-control study were divided into two groups: the TMJD group and control group (CG). The control group had no signs and symptoms of TMJD. The aims of the research and procedures were explained to the participants.

EMG evaluation

The electrical activity of the masticatory muscles was measured using an electromyography (EMG) (Miotool USB, Miotec, Brazil, 14-bit resolution, 2-kHz sampling frequency, 110-dB common mode rejection ratio). Disposable bipolar double differential surface electrodes (Ag/AgCl, Meditrace Kendall-LTP, Chicopee, MA, USA) were used for surface EMG. Signals were amplified with a gain of 2000 (20- 500 Hz filter setting) prior to sampling (2000 Hz). After body hair shaving and skin cleansing with 70% alcohol, pairs of electrodes were fixed above the masticatory muscles (left and right masseter muscles [LM and RM] and left and right temporalis muscles [LT and RT]) (Figure 1). The participants were asked to perform an isometric contraction of the masticatory muscles to find the masseter and temporalis muscles. For the temporalis muscles, electrodes were fixed following a virtual vertical line right above the anterior border of this muscle. Meanwhile, for the masseter muscle, electrodes were fixed 2 cm above the gonial angle. The distance between the electrodes was 20 mm⁽²¹⁾. Reference electrodes were attached to the sternum. The participants were in a sitting position with feet on the ground, knee and hip flexed at 90°, and hands and arms supported on their thighs, with the relaxed body and head at the Frankfurt plane. To register the activity of masticatory muscles, parafilm was bilaterally placed by the examiner between the occlusal surface of the first and second superior and inferior molars. Parafilm was folded 15 times to measure 1.5 cm by 3.5 cm. A maximal voluntary isometric contraction (MVIC) of the masticatory muscle with parafilm (5s repeated three times with one-minute interval between trials) was used for normalization before the



Figure 1. Electrodes placement on masticatory muscles



mastication tasks. Verbal commands were used to motivate participants to reach their maximal jaw strength during MVIC. The participants were trained in the experimental procedures before testing to get used to two tasks of mastication:

A- habitual mastication with parafilm (Neenah, WI, USA) (HM) $\,$

B- non-habitual mastication with parafilm (NHM)

For each task of bilateral mastication, three trials were recorded for 10s with one minute of rest between trials. During habitual mastication (HM), participants were instructed to chew as they habitually do. For NHM (non-habitual chewing cycle), participants were asked to masticate following the metronome cadence (1Hz) set 60 beats per minute to standardize mandibular movement ^(10, 11, 14, 16). The first mastication was always habitual to avoid effects of NHM motion.

EMG processing and analysis

Raw EMG signals were band pass filtered (20 Hz and 500 Hz), rectified using root mean square (RMS), and normalized by the percentage of the maximum value obtained in MVIC for one second between the three repetitions for each subject and muscle. For EMG signal analysis, calculation routine using Microsoft Excel software was implemented to detect the beginning (onset) and the end (offset) of mastication task^(16, 22). The routine automatically searched for the 200-ms epoch with the lowest mean RMS%. This value and its standard deviation were defined as a reference to determine the agonist phase (jaw closing) and antagonist phase (jaw opening) of mastication. Reference values were defined as signal plus three standard deviations of the RMS value. Then the reference value was computed to a transition index (Index (i) = n > (i) + n < (i)) to complete the EMG analysis. If the amplitude of EMG data is lower than the reference value, the transition index is increased (n>(i)), or if the amplitude of EMG data is greater than the reference value, the transition index is decreased (n<(i)). The beginning (onset) and end (offset) of muscle activity are verified by the maximum and minimum values of transition index calculated. This routine detects and uses the filtered EMG signal (band-pass filter with 20-500 Hz bandwidth). The middle of the masticatory cycle was chosen for analysis.

Normalized indexes proposed by Ferrario^(23, 24), the symmetry index (SI%, symmetry of masseter (SM%); symmetry of temporalis (ST%))⁽²³⁾, torque coefficient (TC%)⁽²³⁾ and anterior–posterior coefficient (APC%)⁽²⁴⁾ were used for each muscle during HM and NHM. The symmetry of EMG activity of the muscle pairs has been analyzed by means of the percentage overlapping coefficient (*POC* – unit %). This index showed the total shape of the muscle activation wave as a function of the time, demonstrated by an overlapping percentage of the linear envelope of the RMS normalized during mastication. The normalized values of the amplitude EMG signal from the right and left side (right [R] and left [L] masseter and R and L temporal) were overlapped, and the ratio between the overlapped areas and the total areas between the curves of the R and L muscles RMS was calculated. Symmetrical muscle

pattern should present a ratio close to $100\%^{(23)}$. The TC%⁽²³⁾ was calculate to assessed a possible unbalanced contractile activity of contralateral masseter and temporalis muscles, for instance right masseter and left temporalis, might give rise to a potential lateral displacing component, the Torque Coefficient (%). This index ranges between 0% (no lateral displacing force) to 100% (complete presence of lateral displacing force). The APC% compared the activity between the masseter and temporalis muscles, and it is the ratio between the non-overlapped and the overlapped masseter and temporalis muscle areas of both sides (right; left)⁽²⁴⁾. This index ranged from 0% (no synergy between the masseter and temporalis) to 100% (full synergy between the masseter and temporalis). For EMG signal processing, MATLAB (Version 5.3, MathWorks Inc, Los Angeles, CA, USA) functions were used.

Statistical analysis

The participants were characterized using descriptive statistics (mean, standard deviation, and 95% confidence interval [CI]). Kolmogorov–Smirnov test was applied for data normal distribution, and Levene's test was performed to check for homogeneity of variance. Two-way analysis of variance (ANOVA) test were used to determine differences between groups, mastication and their interactions. The Tukey-HSD test was used for post hoc analysis. The square partial ETA (n_p^2) was used as a measure of effect size (ES) for this analysis. We considered $\eta 2p = 0.0099$, small; $\eta 2p = 0.0588$, medium; $\eta 2p = 0.1379$, large ^{(25).} Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 20.0 for Windows (IBM Corp., Armonk, NY, USA), and a significance level of 5% (p<0.05) was assumed for all procedures.

RESULTS

Clinical characteristics

All TMJD individuals had myalgia with simultaneous diagnosis of disc displacement without reduction without limited opening with arthralgia (23.2±4.0 years) and 27 healthy participants (women without TMJD, 26.4±7.4 years) were included in the study. Groups were not statistically significant different regarding age (p=0.06), weight (p=0.67), and height (p=0.32).

Electromyographic analysis

During jaw antagonist phase (Table 1), the group did not affect muscle activation and no interaction was found between groups and mastication effect. Mastication affected muscle activation. For both groups, the right and left masseters muscles presented more activation during HM compared with NHM type. ST% was the lowest for HM. TC% was significantly increased for HM compared to NHM.

During jaw agonist phase (Table 2), the right masseter muscle of the TMJD group had significantly reduced activation compared to control. Mastication affected muscle activation



Table 1. Average, standard deviation (SD) (RMS%), 95% confidence interval (95% CI) and results of analysis of variance during antagonist phase of jaw for habitual mastication with parafilm (HM) and non-habitual mastication with parafilm (NHM) for control group (n=27) and TMJD group (n=27).

		Control Group	TMJD group	Difference of	Groups	Mastication	Groups versus Mastication	Eta Squared	Observed
		Average (SD)	Average (SD)	mean (95% CI)	p	Р	p		power
RMS% RT	HM	3.10 (2.70)	3.43 (4.04)	-0.33 (2.16;1.50)	0.50	0.32	0.76	0.00	0.09
	NHM	2.56 (1.40)	3.22 (3.52)	-0.66 (2.08;0.76)				0.00	0.10
RMS% LT	HM	2.29 (1.36)	3.79 (4.60)	-1.5 (3.30;0.30)	0.22	0.20	0.66	0.02	0.49
	NHM	2.21 (1.36)	3.01 (3.11)	-0.8 (2.08;0.48)				0.01	0.28
RMS% RM	HM	2.39 (1.71)	3.32 (2.88)	-0.93 (2.19;0.33)	0.32	0.00***	0.46	0.02	0.40
	NHM	1.93 (1.51)	2.49 (2.45)	-0.56 (1.64;0.52)		AxB		0.00	0.19
RMS% LM	HM	2.41 (2.16)	3.68 (4.45)	-1.27 (3.13;0.59)	0.21	0.00***	0.81	0.02	0.50
	NHM	1.75 (1.28)	3.07 (4.55)	-1.32 (3.10;0.46)		AxB		0.01	0.37
ST%	HM	76.83 (18.42)	80.15 (15.90)	-3.32 (12.49;5.85)	0.31	0.01**	0.39	0.01	0.28
	NHM	83.14 (13.03)	83.76 (12.34)	-0.62 (7.38;6.14)		BxA		0.00	0.06
SM%	HM	79.79 (14.97)	82.80 (11.97)	-3.01 (10.23;4.21)	0.43	0.81	0.40	0.01	0.33
	NHM	81.24 (15.95)	81.10 (14.64)	0.14 (8.02;8.30)				0.00	0.05
APC%	HM	72.20 (11.49)	70.06 (13.54)	2.14 (4.55;8.83)	0.17	0.52	0.55	0.00	0.22
	NHM	74. 46 (9.49)	71.15 (16.90)	3.31 (4;10.62)				0.02	0.53
TC%	HM	24.35 (13.85)	20.43 (9.44)	3.92 (2.40;10.24)	0.27	0.00***	0.13	0.02	0.56
	NHM	17.88 (8.39)	18.45 (11.20)	-0.57 (5.84;4.70)		AxB		0.00	0.05

Note: p < 0.05; ** p < 0.01; ***p < 0.001. Control Group (CG), Temporomandibular joint disorder group (TMJD), Right temporalis (RT), left temporalis (LT), right masseter (RM), left masseter (LM), and symmetry index of temporalis (ST%), symmetry index of masseter (SM%), antero posterior coefficient (APC%), torque coefficient (TC%).

Table 2. Average, standard deviation (SD) (RMS%), 95% confidence interval (95% CI) and results of analysis of variance during agonist phase of jaw for habitual
mastication with parafilm (HM) and non-habitual mastication with parafilm (NHM) for control group (n=27) and TMJD group (n=27).

		Control Group	TMJD group	Difference of mean	Groups	Mastication	Groups versus Mastication	Eta	Observed
		Average (SD)	Average (SD)	(93% CI)	p	Р	р	Squareu	power
RMS% RT	HM	44.61 (19.99)	41.96 (16.43)	2.65 (6.87;12.17)	0.33	0.00***	0.32	0.00	0.10
	NHM	58.69 (28.19)	47.79 (14.13)	10.09 (1.80;21.98)		BxA		0.03	0.61
RMS% LT	HM	41.11 (20.05)	44.67 (17.96)	-3.56 (13.71;6.59)	0.83	0.00***	0.07	0.01	0.37
	NHM	56.85 (30.16)	47.56 (15.98)	9.29 (3.58;22.16)		BxA		0.00	0.17
RMS% RM	HM	40.42 (18.70)	37.84 (19.19)	2.58 (7.52;12.68)	0.03*	0.00***	0.00***	0.01	0.28
	NHM	59.50 (26.78)	38.69 (17.31)	20.81 (-8.78;32.83)		BxA	CG (BxA) CG(B) x TMJD(A,B)	0.19	1.00
RMS% LM	HM	36.88 (22.66)	39.15 (19.22)	-2.27 (13.47;8.93)	0.93	0.00***	0.01**	0.00	0.21
	NHM	54.09 (29.99)	43.88 (18.72)	10.21 (13.47;8.93)		BxA	CG(BxA)	0.02	0.42
ST%	HM	88.97 (9.21)	86.41 (14.52)	2.56 (3.12;23.54)	0.74	0.36	0.15	0.01	0.32
	NHM	89.18 (14.26)	90 (9.05)	0.82 (5.55;7.19)				0.00	0.13
SM%	HM	87.47 (12.57)	88.19 (12.79)	-0.72 (3.55;8.01)	0.72	0.32	0.18	0.00	0.05
	NHM	89.86 (9.35)	87.63 (12.15)	2.23 (4.88;4.24)				0.01	0.27
APC%	HM	82.58 (9.51)	82.90 (7.47)	-0.32 (4.88;4.24)	0.32	0.04*	0.02*	0.00	0.06
	NHM	87.23 (6.10)	83 (9.33)	4.23 (-0.02;8.43)		BxA	CG(BxA)	0.05	0.84
TC%	HM	17.48 (8.43)	15.71 (8.84)	1,77 (2.83;6.37)	0.86	0.00***	0.20	0.01	0.40
	NHM	11.54 (5.32)	12.96 (7.89)	-1.42 (5;2.16)		AxB		0.01	0.30

Note:* p < 0.05; ** p < 0.01; ***p < 0.001. Control Group (CG), Temporomandibular disorder group (TMJD), Right temporalis (RT), left temporalis (LT), right masseter (RM), left masseter (LM), and symmetry index of temporalis (ST%), symmetry index of masseter (SM%), antero posterior coefficient (APC%), torque coefficient (TC%).



and NHM type presented more activation for RMS% of right and left temporalis/ masseters muscles and increase balanced contractile activities (APC% index) compared to HM. The TC% was significantly increased for HM compared to NHM.

Interactions were found between group and mastication. For CG, NHM presented significantly more muscle activation compared to HM for right and left masseters muscles and increased APC% index. Also, the right masseter muscle in the control group during NHM presented significantly more muscle activation compared to HM and NHM of TMJD group.

DISCUSSION

The main finding of this study is that habitual mastication and non-habitual mastication pattern differ in masticatory activity either on jaw agonist or in jaw antagonist phase. Habitual mastication presented more variability in surface EMG activity and reduced masticatory recruitment during the agonist phase compared to the other pattern assessed. Non-habitual mastication had less variability in surface EMG activity and presented increase masticatory activity due to a more stable pattern in recruiting muscles. Our findings suggest that NHM has a more coordinating pattern in masticatory muscle activity compared to HM. This knowledge is useful for healthcare professionals to elaborate protocols of assessments for research and clinical practice purpose when patients present impairments to contract muscles.

Patients with TMJD have constraints in the masseter and temporalis muscle recruitment that affect orofacial motor functions ⁽⁴⁾. However, similar to another study ⁽¹⁰⁾ during the inactive phase individuals with TMJD and controls exhibited similar behavior. Women with TMJD in this study had chronic pain for at least six months and the low intensity of TMJD pain during tests might have affected the results, and it could be one reason for similar EMG results between women with and without TMJD.

During the agonist phase, only the right masseter muscle activity of the TMJD group decreased during HM and NHM compared with the control group. Ries et al.,⁽⁹⁾ observed that lower muscle activity under those circumstances may be related to quick susceptibility to fatigue in TMJD compared to the asymptomatic group. Reorganization in masticatory activity in TMJD patients can occur when the stomatognathic system needs to use adaptive processes to maintain the efficient execution of oral motor functions^(3, 5) such as, reduction in muscle recruitment during chewing^(4, 11, 13, 14) to avoid more damage and pain to masticatory tissues^(4, 6) when muscles have problems to contract due to pain. Although this initial adaptation in muscle activity has a short-term benefit, if persist in the long term can result a maladaptation⁽²⁶⁾ and may lead or aggravate dysfunction, pain and disease⁽⁴⁾.

Regarding the comparison between mastication patterns, during the antagonist phase for both groups, the activity of the masticatory muscles was higher for HM compared with NHM. Habitual mastication increased the activity of masticatory muscles during jaw depression movement, thereby suggesting a need for antagonist muscle activation to increase jaw motion control. According to the pain adaptation model⁽⁶⁾, the sensorimotor jaw system and movements affect how pain interacts with muscle activity. Non-habitual mastication pattern presented more muscle activation during the agonist phase for the four masticatory muscles compared to habitual mastication. Also, even more muscle activation of right masseter muscle during NHM in the asymptomatic group when compared to both conditions assessed in TMJD group. It means that this type of mastication has a similar pattern of occlusion and the TMJD group has a different way to recruit muscles under these circumstances. Standardized mastication imposes time constraints to chew with the jaw moving according to metronome pace. Voluntary contractions require greater involvement of voluntary motor cortex to modulate the central pattern generation and perform standardized chewing in comparison to the semiautomatic movements in free chewing which may require less cortical involvement⁽²⁷⁾. A recent experimental pain study assessed the effects of isotonic versus hypertonic saline infusion on masticatory muscle during habitual and standardized mastication and found the experimental procedures have different effects on muscle activity independent of whether the muscle was an agonist or antagonist in the task, but did not make comparisons between tasks ⁽²⁸⁾. The authors argued that the differences in muscle activity in the same task also may reflect the differing mix of somatosensory afferent and descending motor inputs to the motoneurons from muscles during the different tasks, which are likely to differentially modulate the effects that nociceptive inputs have on the neural networks driving these motoneurons (28). Muscle pain and movements involves a diversity of changes in muscle activity from subtle redistribution of activity within and between muscles to complete or relative avoidance of movement and the relationship between pain and movement is complicated by the reality that movement can both reduce or increase pain and also is influence by biological, psychological, and social factors (26).

This study contributes to show the physiological aspects of contractile activities of masticatory muscles during agonist and antagonist phases of the mastication cycle in TMJD pain individuals to reach the neuromuscular balance during these oral motor tasks. Most of published researches do not characterize the clinical aspects of masticatory activity into jaw phases: agonist and antagonist period. The implication of our findings suggests that non-habitual mastication should be used when muscles have impairments to contract to optimize masticatory efficiency because it has a more stable and coordinated motor control pattern to recruit masticatory muscle during chewing.



The groups had similar indexes of symmetry during the agonist and antagonist period. However mastication pattern affected the symmetry and coordination of masticatory muscles. Reduced temporalis symmetry during the antagonist phase for habitual mastication suggests that for this movement pattern, groups have more problems in adjusting their jaw position during free mastication because the temporalis muscles act to control jaw position⁽²⁹⁾. Studies show that TMJD pain patients have uncoordinated masticatory activities during chewing indicating impaired oral motor function^(3, 4, 11). Habitual mastication seems to increase the uncoordinated aspect of masticatory activity during chewing in TMJD. The pattern observed in our study of a more unbalanced contralateral activity of masseter and temporalis muscles (TC%) during HM and the increase synergy among masticatory muscles (APC%) for non-habitual mastication in the agonist phase suggests that habitual mastication is more affected by jaw motion and is not related exclusively to any muscle disorder due to TMJD. This result supports our expectations that habitual and non-habitual mastication patterns differ in muscle recruitment and coordinating physiology of masticatory activities.

A recent systematic review concludes that there is evidence for craniocervical postural misalignment in individuals with TMJD⁽³⁰⁾; however, it is not possible to associate our results to any postural misalignment because postural assessments were not enrolled in the initial design of this study. A limitation of the current study was to include only women because of the higher prevalence of signs and symptoms in this population. We do not know if the same results would be observed in men. Another limitation was not to measure if the performance of any chewing task was more comfortable than the other.

CONCLUSION

The study provides a guide about how pain interacts with movements into the contractile activity of masticatory muscles. Habitual and non-habitual mastication differ in masticatory activity during jaw agonist and antagonist phase and TMJD individuals presented a different way to recruit muscles under these circumstances. Non-habitual mastication has a more coordinating and stable motor pattern compared to the habitual mastication pattern.

AUTHOR'S CONTRIBUTIONS

CEF and LGKR were responsible for the design of the study and guidance of procedures and manuscript; CEF, LGKR and MDG were responsible for volunteer recruitment, data collection and writing of the manuscript; DLM, LPS and LM were responsible for data analysis and writing the manuscript.

CONFLICT OF INTEREST

nothing to declare.

AUTHORS DETAILS

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