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The Reliability of MyotonPRO in Assessing Masseter Muscle Stiffness and the Effect of Muscle Contraction

Authors' Contribution:

Study Design A

Data Collection B

Statistical Analysis C

Data Interpretation D

Manuscript Preparation E

Literature Search F

Funds Collection G

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Background: Temporomandibular disorders (TMD) are accompanied by masticatory muscle-related pain, making it meaningful to assess the stiffness of the masticatory muscles. The present study investigated the intra- and inter-operator reliabilities of MyotonPRO for assessing the elasticity of masseter muscles, to determine minimal detectable changes, and to quantify changes in stiffness from conditions of relaxation to maximal contraction.


Material/Methods: Twenty healthy subjects (10 men and 10 women) were recruited. The stiffness of their masseter muscles was quantified with MyotonPRO in both relaxed and maximal contraction conditions. Two experienced operators (A and B) measured stiffness on the same day, and operator A repeated this procedure 5 days later.

Results: Intra-rater reliability was good (ICC=0.78) and inter-operator reliability was excellent (ICC=0.95) for assessing masseter muscle stiffness with MyotonPRO. The mean stiffness of the masseter muscle on the dominant side was 369.5 N/m under relaxed conditions and 618.3 N/m at maximum bite force, an increase of 67.4%. Stiffness on the dominant and non-dominant sides did not differ significantly under both conditions ($P>0.05$).

Conclusions: MyotonPRO is a reliable method for quantifying the stiffness of the masseter muscle and monitoring its changes under different contraction conditions.

MeSH Keywords: **Elasticity • Isometric Contraction • Masseter Muscle • Reproducibility of Results**

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Background

Temporomandibular disorders (TMD) are common conditions, characterized by pain symptoms and associated with abnormalities in the masticatory muscles and temporomandibular joint (TMJ) [1]. Although 60% to 70% of the normal population have been reported to experience TMD, only around 15% are conscious of their TMD symptoms [1,2]. As a primary cause of masticatory dysfunction, TMD affects activities of daily life and, in particular, leads to psychological problems [3,4]. Efforts to prevent or ameliorate TMD may therefore improve patient quality of life.

Masticatory muscles play an important role in maintaining a balance in masticatory function. Moreover, bite forces influence the masticatory muscles to improve chewing functions [5]. Among these masticatory muscles are the masseter muscles, which help stabilize the grinding path during chewing [6], suggesting that bite forces may be associated with the masseter muscles during mastication. To our knowledge, no study to date has quantified changes in masseter muscle stiffness from conditions of relaxation to maximum bite force. Understanding of TMD may be improved by analyzing changes in the biomechanical properties of the masseter muscles in response to different conditions. Masticatory patterns differ in subjects with and without TMD, in that subjects with TMD frequently adopt a chronic unilateral chewing pattern [7]. Because equal bilateral use of the masticatory muscles during chewing can maintain the balance of masticatory function, one of the objectives of the present study was to quantify whether the biomechanical properties of masseter muscles differ on the 2 sides.

TMD is frequently accompanied by masticatory muscle pain [8], which is frequently diagnosed subjectively by physician palpation. Palpation, however, cannot assess modulations in the biomechanical properties of masseter muscles. The biomechanical properties of these muscles can be quantified by assessing fatigue, by surface electromyography (EMG), and by MyotonPRO measurements of muscle stiffness. Although alterations in masseter muscles have been evaluated by EMG [9,10], this method has limitations, as the electrical signal is easily affected by the experimental environment [11]. MyotonPRO is a convenient, portable device to quantify muscles stiffness, which can overcome the limitations of EMG. In previous studies, we used MyotonPRO to quantify the biomechanical properties of skeletal muscles, including the gastrocnemius [12] and upper trapezius [13] muscles. More importantly, we found that the stiffness of the gastrocnemius muscle and the Achilles tendon, as measured by MyotonPRO, correlated significantly with the shear modulus as quantified by shear wave elastography (SWE) [14]. These findings suggested that the MyotonPRO can quantify the stiffness of the masseter muscles under conditions

of relaxation and maximal contraction, as well as the difference between them.

The aims of this investigation were to (1) determine the intra- and inter-operator reliabilities of quantifying masseter muscle stiffness and determine minimal detectable change; (2) quantify the change in stiffness from a relaxed state to maximum bite force; and (3) determine the difference in masseter muscle stiffness on both sides.

Material and Methods

Ethics

The study protocol was approved by the Human Subject Ethics Committee of the Luoyang Orthopedic Hospital of Henan Province (KY 2019-001-01) and conformed to the principles of the Declaration of Helsinki. Each subject provided written informed consent before the tests and was informed of all experimental procedures.

Participants

Twenty healthy participants, 10 men and 10 women, with no history of TMD were recruited. Based on 3 repeated measurements in each subject, an $\alpha=0.05$, an 80% power, $P_{o(ICC)}=0.5$, and $P_{i(ICC)}=0.8$, the minimal sample size for reliability analysis was calculated to be 14.4 subjects [15], indicating that 20 subjects were sufficient for reliability analyses. The demographic characteristics of all study subjects, including age, height, weight, and body mass index (BMI), were recorded.

Equipment

MyotonPRO (Muomeetria Ltd., Tallinn, Estonia) is a hand-held device used to quantify muscle stiffness. This device operates by generating a mechanical impulse on the skin overlying the muscle being assessed, followed by MyotonPRO measurements of the mechanical oscillations of muscles produced by the mechanical impulse [16]. Muscle stiffness was measured as newtons/meter (N/m). This method can determine the resistance of the muscle to deforming forces or muscle stiffness.

Procedures

All participants were examined in the department of physiotherapy, in which the temperature was maintained around 25°C. Each subject sat on a chair and was allowed to rest for 5 minutes with their heads held in a natural position. The examination sites were marked at the highest site of the masseter muscle during jaw-clenching with maximum force. Stiffness of both sides of the masseter muscles was quantified by MyotonPRO



Figure 1. Measurements of masseter muscle stiffness.

in both relaxed and maximum bite force conditions. All measurements were taken 3 times and their means were calculated for further analysis.

Reliability tests were performed on the right sides of masseter muscles. Tests were performed by 2 experienced operators (A and B). Following each evaluation, the site marked on each subject was cleaned, and the raters were blinded to the results during the tests. To determine intra-operator reliability test, operator A evaluated masseter muscle stiffness on 2 occasions 5 days apart. To determine inter-operator reliability, operators A and B evaluated muscle stiffness on the same day, with a 30 min rest period between evaluations. Figure 1 shows MyotonPRO measurements of masseter muscle stiffness.

Statistical analysis

All statistical analyses were performed using SPSS version 21.0 statistical software (SPSS Inc, Chicago, IL), with a $P < 0.05$ defined as statistically significant. Intra- and inter-rater reliability were determined by calculating intraclass correlation coefficients (ICC) with 95% confidence intervals (CI), using a 2-way mixed effects model and a 2-way random effects model. Standard error of measurement (SEM) was calculated as standard deviation $\times (1 - \text{ICC})^{1/2}$ and minimal detectable change (MDC) as $1.96 \times \text{SEM} \times (2)^{1/2}$. Systematic error [17] and the degree of agreement about reliabilities were determined using Bland-Altman plots. Differences in masseter muscle stiffness on the 2 sides, as well as differences in stiffness during relaxation and maximum bite force, were compared with paired t -tests.

Table 1. Demographic characteristics of the study subjects. SD, standard deviation.

	N=20	Mean±SD
Age (years)		25.40±11.55
Weight (kg)		62.50±12.54
Height (m)		1.69±0.08
Body mass index (kg/m ²)		21.77±3.38

SD – standard deviation.

Results

Demographic information

The demographic characteristics of the 20 study subjects, including their mean±SD age, weight, height, and BMI, are presented in Table 1.

Intra- and inter-rater reliabilities

Reliability tests were performed by measuring masseter muscle stiffness on the dominant side while in a relaxed condition using the MyotonPRO (Table 2). Mean stiffness assessed by operators A and B on the same day was 369.5 N/m and 387.4 N/m, respectively, whereas mean stiffness determined by operator A 5 days later was 350.2 N/m. Intra-operator reliability was good, with an ICC of 0.78, whereas inter-operator reliability was excellent, with an ICC of 0.95. All minimal detectable changes were > 58.8 . Bland-Altman plots showed that, for the intra-reliability test, the mean difference was 17.95 N/m and the 95% limits of agreement (LOAs) were -147.9 to 183.8 N/m (Figure 2A); whereas, for the inter-reliability test, the mean difference was -19.2 N/m, and the 95% LOAs were -92.9 to 54.5 N/m (Figure 2B).

Differences in masseter muscle stiffness under different conditions

The MyotonPRO can also be used to quantify differences in masseter muscle stiffness under different conditions (Figure 3). The mean stiffness of the masseter muscle on the dominant side was 369.5 N/m under relaxed conditions and 618.3 N/m at maximum bite force, an increase of 67.4% (Table 3).

Differences in masseter muscle stiffness on the right and left sides

Mean masseter muscle stiffness under relaxed conditions was 369.5 N/m on the right side and 347.5 N/m on the left side, a difference that was not statistically significant ($P > 0.05$) (Table 3). At maximum bite force, mean masseter muscle

Table 2. Intra- and inter-rater reliabilities for assessing masseter muscle stiffness by MyotonPRO.

	Mean±SD	MDC	SEM	Reliability (95% CI)
Rater A in test 1	369.5±94.9	58.8	21.2	Intra-operator: 0.78 (0.46~0.91)
Rater B	387.4±106.7	66.1	23.9	
Rater A in test 2	350.2±89.5	116.3	41.9	Inter-operator: 0.95 (0.89~0.98)

CI – confidence interval; SEM – standard error of measurement; MDC – minimal detectable change; SD – standard deviation.

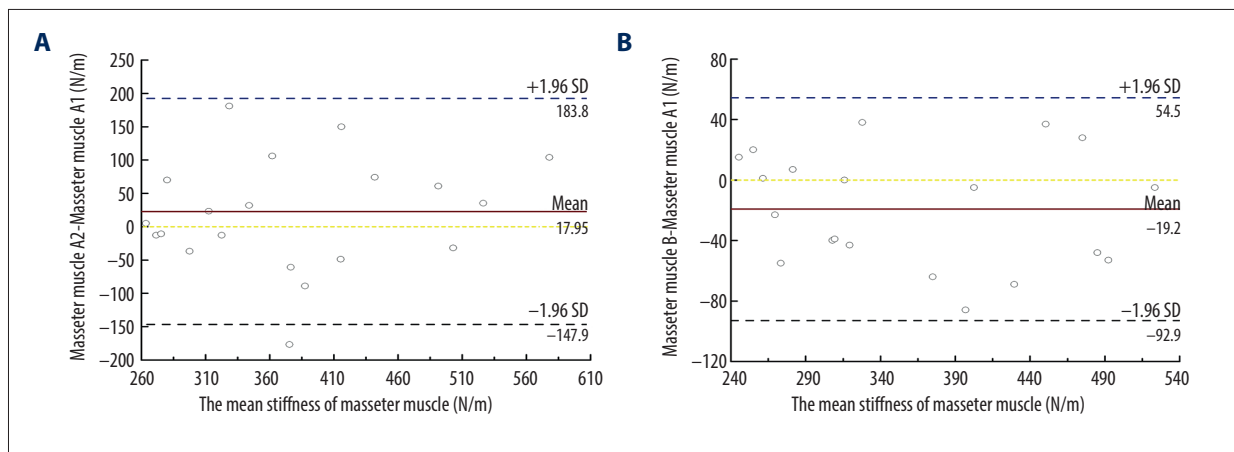


Figure 2. Bland-Altman plots of (A) intra-operator and (B) inter-operating reliabilities for assessing masseter muscle stiffness.

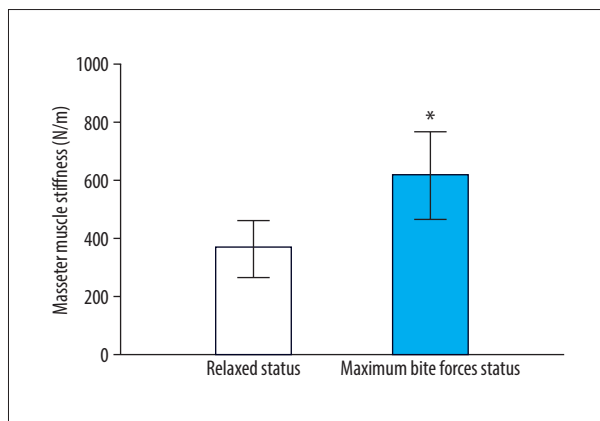


Figure 3. Mean±standard deviation masseter muscle stiffness under conditions of relaxation (white bar) and maximum bite force (blue bar). * $P<0.05$.

stiffness was 618.3 N/min on the right side and 607.1 N/m on the left side ($P>0.05$).

Discussion

This study used the MyotonPRO device to explore the intra- and inter-observer reliabilities of measuring masseter muscle stiffness, the difference in stiffness between the right and left sides, and the difference in stiffness under different conditions in healthy subjects. The MyotonPRO showed excellent intra- and inter-operator reliability in assessing masseter muscle stiffness and found no difference between the right and left sides. Additionally, muscle stiffness was greater at maximum bite force than under relaxed conditions.

Table 3. Masseter muscle stiffness on both sides during conditions of relaxation and maximum bite force.

	Relaxation (Mean±SD)	Maximum bite force (Mean±SD)	P
Non-dominant side	347.5±83.5	607.1±158.7	0.000
Dominant side	369.5±94.9	618.3±150.7	0.000
P	0.102	0.675	

P values for the difference between conditions of relaxation and maximum bite force and for the difference of each on the dominant and non-dominant sides were calculated by paired t-test, with P values <0.05 shown in **bold**.

Intra- and inter-rater reliabilities

To our knowledge, this study is the first to assess the reliability of MyotonPRO measurements of masseter muscle stiffness. We found that the intra-operator reliability was good and the inter-operator reliability was excellent, indicating that the MyotonPRO was reliable in assessing masseter muscle stiffness in a healthy population. The MyotonPRO has also been shown reliable in evaluating skeletal muscle stiffness. For example, the Myotonometer showed good intra-observer reliability (ICC >0.72) in measurements of biceps and triceps brachii muscle stiffness in subjects with subacute stroke, but inter-observer reliability was not evaluated [18]. Moreover, the MyotonPRO showed high intra-observer reliability in measurements of quadriceps muscle tone in older subjects, with ICCs ranging from 0.7 to 0.9 [19]. The MyotonPRO also showed excellent intra- and inter-observer reliabilities in determinations of the test-retest reliability of upper trapezius stiffness, with both ICCs being 0.97 [13].

Other methods have been used to quantify masseter muscle stiffness in patients with masticatory myofascial pain. For example, SWE showed good reliability, with an ICC >0.75 [20]. The difference in reliability between previous studies and ours was likely due to differences in assessment techniques. We also found that the MDC, an indirect measure of reliability, was >58.8 N/m, with a smaller MDC indicating higher reliability. Our findings indicate that MyotonPRO has certain advantages in quantifying masseter muscle stiffness.

We found that inter-rater reliability was better than intra-rater reliability. The difference may have been due to the 5-day interval between intra-operator assessments. Reliability may have been affected by many factors during those 5 days, including differences in exercise and minor changes in the experimental environment. Additionally, the Bland-Altman plots, which can indicate the degree of consistency, showed good reliability [17]. The mean difference in intra-observer measurements was 17.95 N/m (95% LOA -147.9 to 183.8 N/m), comparable to the mean difference in inter-observer measurements (-19.2 N/m; 95% LOA -92.9 to 54.5 N/min).

Masseter muscle stiffness under different conditions

Mean masseter muscle stiffness, as evaluated by MyotonPRO, was 369.5±94.9 N/m under relaxed conditions, but was 618.3±150.7, or 64.7% higher, at maximal contraction, with an MDC >58.8 N/m. Bite force is associated with the biomechanical characteristics of the masticatory muscles, including the masseter muscle [5,21]. The masseter muscles play an important part in balancing masticatory function [22]. The 64.7% increase in masseter muscle stiffness may enhance the stability of masticatory function during the modulation of bite forces. Moreover, changes in status were shown to alter other biomechanical properties of

the masseter muscles. For example, ultrasound examination of children with unilateral posterior cross-bite showed that masseter muscle thickness correlated positively with bite force, with maximal bite force increasing thickness 23.1% [23]. EMG showed that fatigue was greater in subjects with TMD than in healthy controls, with the duration of masticatory muscle contraction affecting the activity of these muscles [24]. SWE also showed that masseter muscle stiffness was significantly greater in subjects with TMD than in control subjects [20]. Myoton-3 quantitation of contracted and relaxed facial muscles yielded findings similar to ours, with muscles being stiffer during contraction [25].

Taken together, these results indicate that the biomechanical properties of masseter muscles are influenced by the state of contraction. These findings may enable better understanding of the relationships between masticatory muscles and masticatory function. MyotonPRO evaluation of masseter muscle stiffness in relaxed conditions and at maximum bite force may be a reliable reference for future studies.

Difference in masseter muscle stiffness between the right and left sides

Interestingly, masseter muscle stiffness did not differ significantly on the right and left sides, both under conditions of relaxation and at maximum bite force. Physiologically, the 2 sides of the masseter muscle should be equally involved in maintaining masticatory function. Similar findings were observed using other techniques. For example, SWE evaluation of healthy subjects showed no difference in masseter muscle stiffness on the 2 sides [26]. Additionally, masseter muscle thickness on both sides was found to be similar [27]. Unilateral chewing has been shown result in differences in the thickness and activities of the masseter muscles on the 2 sides [28], and unilateral mastication was associated closely with TMD [29]. Our findings suggested that chewing in healthy populations is not unilateral, preventing the development of TMD.

Limitations

This study had several limitations. First, only healthy subjects were recruited, suggesting the need to evaluate masseter muscles in patients with pathological conditions. In addition, many factors may have affected assessments of masseter muscle stiffness; including the pressure needed to hold the MyotonPRO to the masseter muscle and muscle activity, which may have been affected by the timing or duration of exercise. Furthermore, the MyotonPRO evaluated stiffness at 1 point of each masseter muscle, but this point may not have been representative of overall muscle stiffness. Finally, the maximal contraction status of the masseter muscle was defined subjectively. Additional studies are therefore necessary to quantify the maximal contraction of the masseter muscles.

Conclusions

In conclusion, MyotonPRO is a novel technique with high reliability in quantifying masseter muscle stiffness under both relaxed conditions and at maximum bite force. The finding that stiffness was similar on both sides provides a reference for future studies.

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Conflicts of interest

None.