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Evaluation of Masseter Muscle Dimensions Through Ultrasonography

Evaluación de las Dimensiones del Músculo Masetero Mediante Ultrasonido

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ABSTRACT: The objective this study was to determine mean values of masseter muscle thickness in male and female, at rest and contraction, in healthy individuals.At first, 102 questionnaires were distributed between students. Thirty patients were selected, 15 male and 15 female, according to the inclusion criteria, excluding all individuals with symptoms of temporo-mandibular dysfunction or syndromes with craniofacial disorders. Masseter muscle thickness was determined bilaterally, at rest and contraction. As a reference point for the measurement of thickness (axial plane), the posterior prolongation of the labial commissure was used. Normality values of the masseter muscle in females were 11.75 ± 1,14 mm in contraction and 10.19 ± 1.04 mm in relaxation; and in the male were 13.95 ± 1.62 mm in contraction and 11.64 mm ± 1.68 mm in relaxation.The results obtained are in agreement with those described in the literature. Demonstrating the accuracy and reproductibility of the ultrasound if a protocol is applied, mainly aimed at controlling the pressure applied by the transducer.

KEY WORDS: masseter muscle, ultrasound, ecography, thickness, craniofacial morphology.

INTRODUCTION

The masseter muscle (MM) has relevance in kinematics, mandibular growth, and facial aesthetics (Frugone *et al*., 2010; Oliveira *et al*., 2015). It presents a complex internal structure, with numerous aponeuroses and two main portions: One superficial and another deep; formed since muscle fibers with different orientations, responsible by creation of numerous force vectors with different function in the mandibular movement (Kubo *et al*., 2006).

The development and functioning of the MM occur in an organized and planned manner, according to the functional and environmental stimuli to which it is subject (Palinkas *et al*., 2010). Thus, the study of this muscular structure is fundamental for the morphofunctional characterization of the craniofacial complex, which may include the morphological, histological study and eventual electrical activity (Oliveira *et al*.).

The evaluation of the structure of muscle fibers is possible using imaging methods such as Computed Tomography (CT), Magnetic Resonance (MR) and Ultrasound, this, given its viability in the dynamic and static study of the musculature, presents itself as the method of excellence for assessing MM thickness (Oliveira *et al*.; Close *et al*., 1995). The ultrasound is an accessible, low-cost, non-invasive test, capable of ensuring uncomplicated visualization of facial structures, particularly useful in assessing the transversal dimension of MM invivo (Oliveira *et al*.; Bakke *et al*., 1992; Close *et al*.). Since that strict protocol is followed, reproducibility and viability are ensured for muscle measurement, which allows the acquisition of objective and easily comparable parameters (Oliveira *et al*.; Satirog˘lu *et al*., 2005). MM thickness has been widely studied (Kiliaridis & Kälebo, 1991; Bakke *et al*.; Raadsheer *et al*., 1996; Emshoff *et al*., 2003; Satirog˘lu *et al*.; Georgiakaki *et al*., 2007), however, heterogeneity

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of the measures observed in the literature could be minimized by subdividing the muscle into segments and standardizing the number of measures for a morphometric analysis (Reis Durão *et al*., 2017).

Dysfunctional contraction mechanisms are responsible for modifying the muscle structure, including its thickness, through edema and / or muscle hypertrophy (Aldemir *et al*., 2013). Excessive load generates an imbalance in the various mechanisms of the craniofacial complex through a transient inflammatory condition, or a definite increase in muscle thickness (Kiliaridis *et al*., 2010; Ariji *et al*., 2010).

Several authors used the ultrasound to understand the correlation of MM dimensions with factors such as age, sex, bite force, facial type, arcade shape and occlusion, reinforcing the morphofunctional importance of this muscle, as well as its relevance in maintaining integrity of the stomatognathic system (Bakke *et al*.; Satirog˘lu *et al*.; Palinkas *et al*.). The quantification of the masseter thickness is possible due to its superficial location (Ariji *et al*., 2004) and it becomes crucial for the evaluation of the masseter muscle morphology and consequent detection of asymmetries and hypertrophy.The data obtained in different investigations are important for the study and treatment of different pathological conditions inherent to the stomatognathic system, allowing the creation of clinical protocols based on values reference for each sex, enhancing the diagnosis and treatment of the respective conditions (Frugone *et al*.).

Thus, the objective of the present study is to determine the thickness of the MM, through ultrasound, correlating with lifestyle and nutritional habits for both sexes.

MATERIAL AND METHOD

Sample selection: The study was approved by the Ethics Committee of the Faculty of Dental Medicine of the University of Porto (Approval nº 22). One hundred and two volunteer students from the same university were approached. Each participant consented to participate in the study by signing the informed consent. Subsequently, an intra and extra-oral evaluation was carried out with the respective data collection form.
To guarantee the acquisition of the normality standard for MM thickness, all individuals who presented loss of dental parts, except third molars and / or premolars, prosthesis or orthodontic treatment, history of orthognathic treatment, facial trauma, were excluded, mandibular fracture, injection of botulinum toxin in the face and individuals with a history of neuromuscular diseases, craniofacial disorders, and symptoms and / or temporomandibular disorders.

In the second phase of data collection, all previously described parameters were applied, 30 individuals were selected, 15 females and 15 males.

Ultrasound analysis. The ultrasound determination of the MM thickness was assessed by a radiologist doctor with 30 years of experience and a maxillofacial radiologist with 15 years of experience. In cases of disagreement between the observers, consensus was reached by discussion. The ultrasound used were performed at the GE Healthcare (unit of the General Electric Company, United Kingdom), with a 12 mm linear type transducer. The ultrasound evaluation was performed in an appropriate room for diagnostic imaging exam, with low light intensity.

Patient position. All participants remained seated in a supine position - Frankfurt plane parallel to the floor, without any means of fixing the head, with their eyes open and their hair removed from the face. The location of the MM was detected by instructing the patient to perform strong maximum intercuspation with palpation of the anterior margin, followed by moving the linear transducer for confirmation. Once the muscle was located, a water-based gel was applied to the area between the posterior and anterior edge of the mandible branch, keeping the transducer in a position perpendicular to the skin, with reduced pressure (Fig. 1).

Fig. 1. Patient position for ultrasound examination and determination of muscle location.
A. Patient's supine position. Note the head ‘position.
B. Location of the masseter muscle through clinical examination - muscle palpation.

The images were taken bilaterally under the two conditions described, at rest and maximum intercuspation (maximum contraction of the MM). During image acquisition, the patient was asked to slightly rotate his head to the side contralateral to the one under analysis.

To determine the thickness of the muscle (in the axial plane), the posterior extension of the labial commissure was used as an anatomical reference (Fig. 2).

Fig. 2. Position of the linear transducer during ultrasound image acquisition.

Measurements. The measurements were obtained in real time and directly on the image, to the nearest 0.1 mm (Figs. 3 and 4).

Statistical analysis. The descriptive analysis of the data was performed using Excel® software (Version 2016; Microsoft, Redmond, Washington - USA) and the statistical software SPSS® (Statistical Package for Social Sciences 24.0 for Windows, SPSS Inc., Chicago, Illinois - USA), also used for statistical analysis.

The description of the data is presented through an analysis of percentages for the variables sex, lifestyle and nutritional habits and determination of the mean ± standard deviation for the continuous variables, age, and height.

In the statistical analysis of the cases, a significance level of a = 0.05 was defined for all tests. Intraobserver and interobserver reliability were evaluated by Kappa statistics. The Shapiro-Wilk test was applied to assess the normality of the values obtained in the two groups under study.

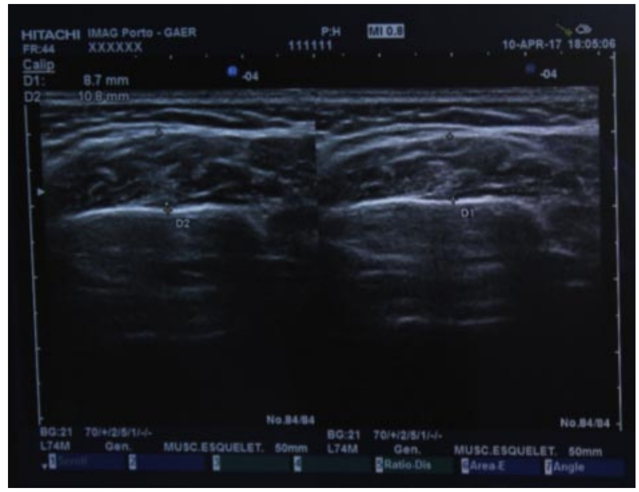

Fig. 3 Measurement in contraction and rest.

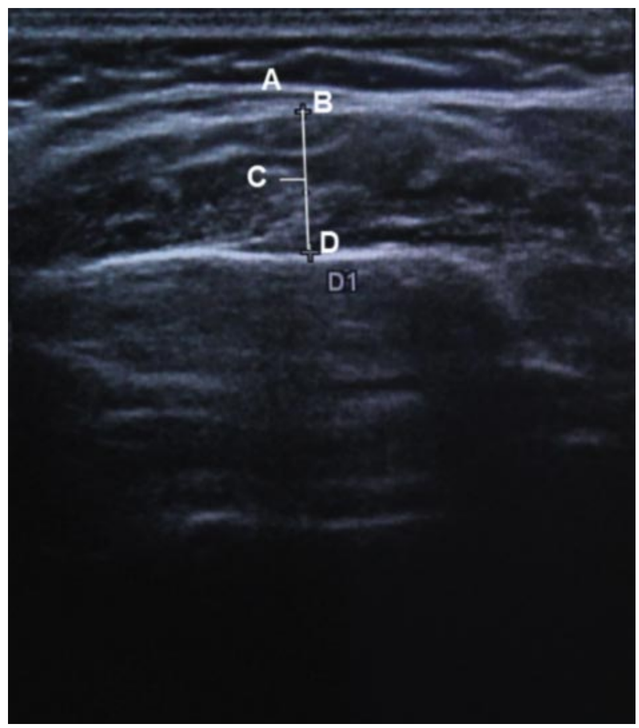

Fig. 4. Image obtained by ultrasound.
A - White shadow represents the most superficial structure - skin.
B - Upper limit represented by a white trace representing the muscular fascia.
C - Heterogeneous dark area corresponds to the transversal dimension of the muscle.
D - Lower limit represented by a white image corresponds to the lateral surface of the mandibular ramus.

RESULTS

Descriptive analysis of the population sample. Intra- and inter-observer correlations were found excellent for all the evaluations (0.91 and 0.85, respectively). Of the 60 ultrasounds performed, 30 correspond to women and 30 to men. The ages of the patients included in this study at the time of data collection vary between 19 and 30 years old, with an average of 23.5 ± 2.3 and a median of 23 years. The graph in Figure 5 describes the distribution of the sample population by age group according to sex.

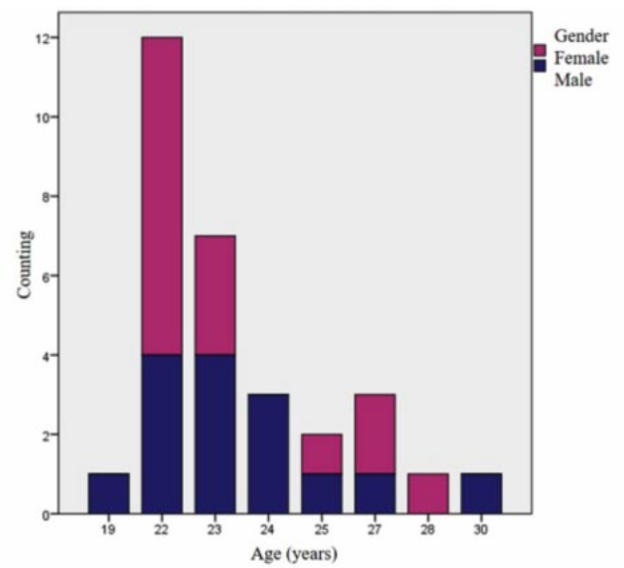

Fig. 5. Representative graph of the sample population by gender and age

The heights of the individuals included in this study at the date of data collection vary between 157 cm and 195 cm, with an average of 169.87 ± 8.20 cm. The Fig. 6 describes the distribution of the sample population by height according to sex (Fig. 6).

Regarding nutritional habits, 26.7 % of individuals, on average, have seven "Meals with meat per week". Figure 7 represents the distribution of the number of meat meals per week.

70 % of the individuals in this study presented "Lifestyle: Active" and 30 % pf the individuals presented "Lifestyle: Pasive".

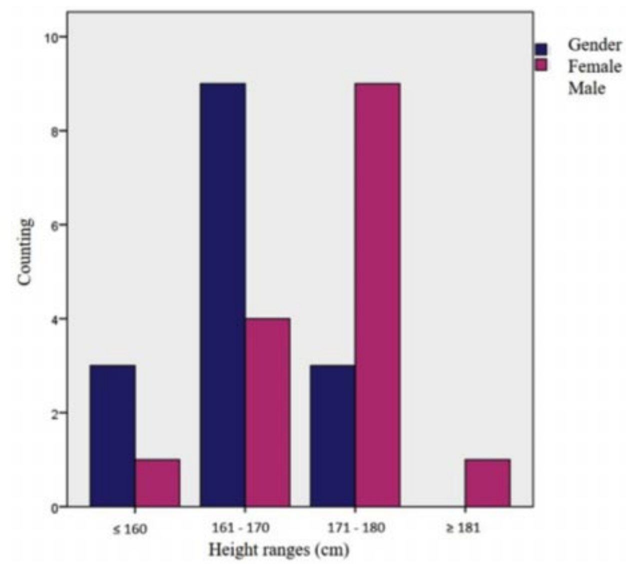

Fig. 6. Representative graph of the sample population by gender and height

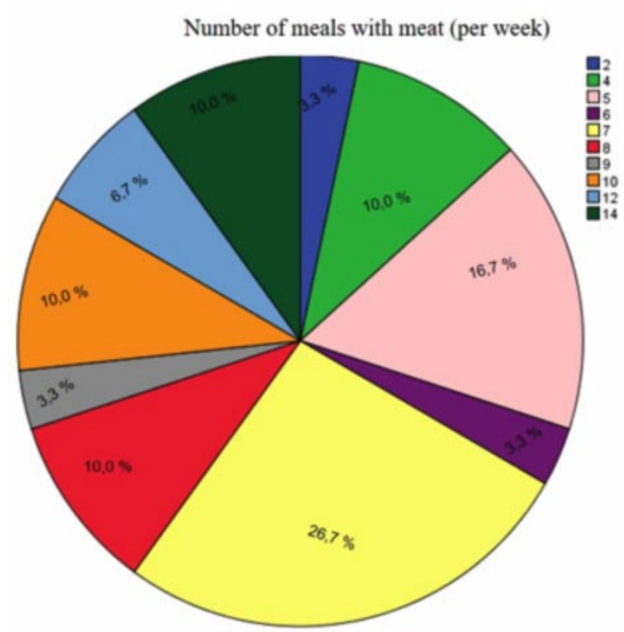

Fig. 7. Representative graph of the distribution of "Meals with meat per week", in percentage (%)

Statistical analysis of the data

MM thickness correlating sexes. The normality statistical was previously evaluated, having obtained a value of P = 0.650 for the thickness of the MM in contraction and a P = 0.794 for the thickness of the MM in relaxation. Since P>a, the values are considered statistically significant, and therefore normality is accepted. The mean values of MM thickness for females were 11.75 ± 1.14 mm in contraction and 10.19 ± 1.04 mm in relaxation (Fig. 8, trend of normality of values obtained for females).

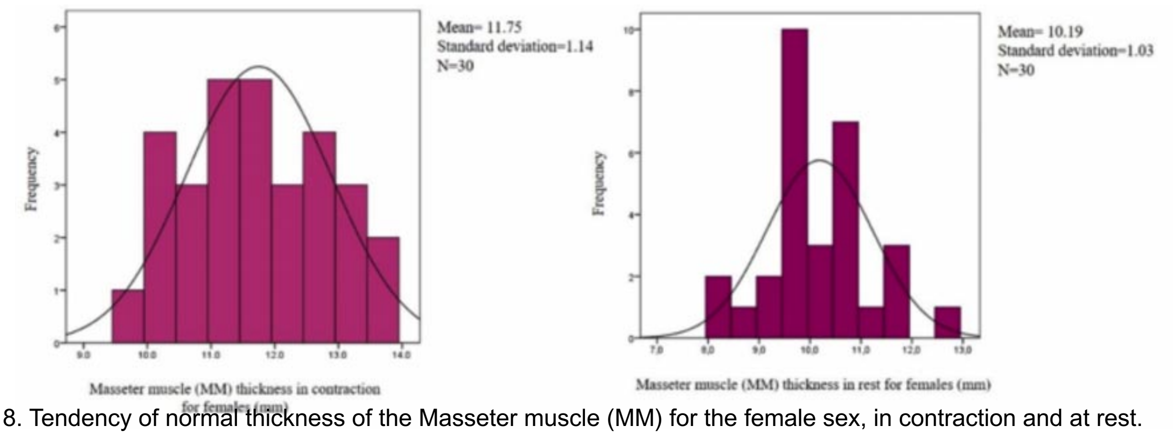

Fig. 8. Tendency of normal thickness of the Masseter muscle (MM) for the female sex, in contraction and at rest.

Table I Masseter muscle thickness values in females obtained by ultrasound.

Indivídual (female sex)	Contraction thickness – right side (mm)	Contraction thickness – left side (mm)	Thickness at rest - right side (mm)	Thickness at rest - left side (mm)
1	12.8	13.5	10.2	9.8
2	11.7	13.4	9.5	11.9
3	10.7	12.1	9.0	11.5
4	11.2	11.0	9.9	10.9
5	10.3	11.8	9.5	9.9
6	13.3	13.9	10.6	12.8
7	11.6	11.8	9.8	10.7
8	11.3	11.8	9.8	10.7
9	10.9	11.5	8.4	9.4
10	10.2	12.7	9.6	11.1
11	12.6	12.9	11.7	10.0
12	11.2	11.4	9.5	10.7
13	13.3	10.4	10.4	8.2
14	12.2	9.7	10.9	10.9
15	10.8	10.0	8.7	9.9

Table II Masseter muscle thickness values in males obtained through ultrasound.

Indivídual (male sex)	Contraction thickness - right side (mm)	Contraction thickness - left side (mm)	Thickness at rest - right side (mm)	Thickness at rest- left side (mm)
1	14.6	13.5	12.1	10.8
2	14.4	15.6	13.4	13.0
3	12.8	12.7	10.3	10.7
4	15.9	14.9	14.2	13.2
5	12.8	12.7	10.5	10.1
6	17.8	13.6	15.6	13.0
7	12.9	9.8	12.2	8.4
8	15.7	13.1	11.0	10.8
9	15.9	13.1	11.0	10.8
10	12.1	13.7	9.7	10.8
11	16.9	14.9	14.5	12.6
12	13.1	12.5	9.4	10.1
13	14.3	13.2	12.0	10.5
14	14.8	15.2	13.5	13.3
15	13.1	13.0	10.6	11.0

Likewise, a value of P = 0.198 was obtained for the thickness of the MM in contraction and a P = 0.222 for the thickness in relaxation. For males, the mean MM thickness was 13.95 ± 1.62 mm in contraction and 11.64 ± 1.68 mm in relaxation (Fig. 9, trend of normality of values obtained for males.

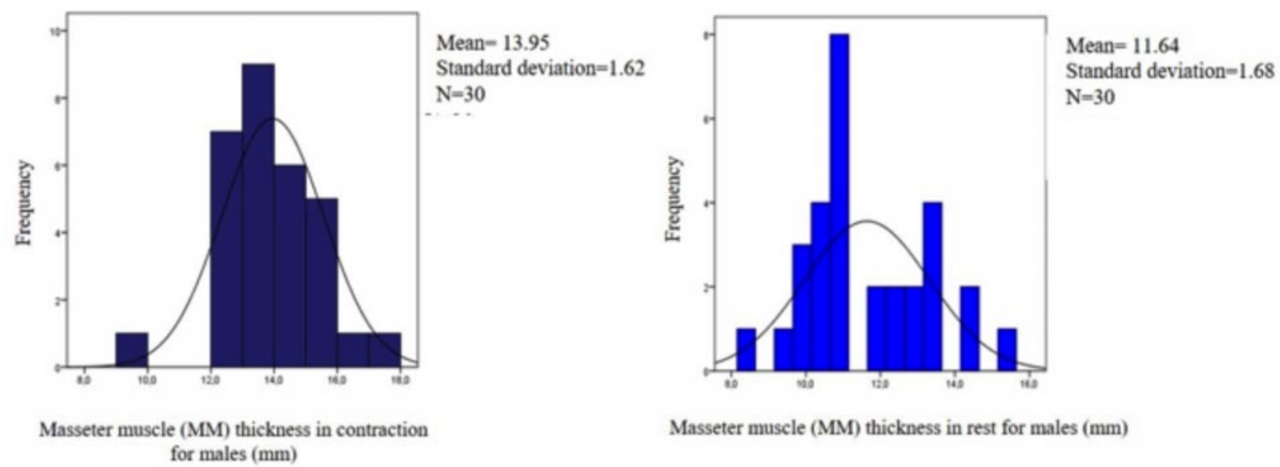

Fig. 9. Tendency of normal thickness of the Masseter muscle (MM) for the male sex, in contraction and at rest.

DISCUSSION

This research investigated the thickness of the MM using ultrasound and its relation to both sexes based on measuring protocols described in the literature and its association with nutritional habits and active or sedentary lifestyle.

In the literature, it is described that ultrasound is sufficiently sensitive to detect changes in the order of 10 to 20 % in the thickness of the MM, determining morphological changes in vivo and in real time (Oliveira *et al*.). In addition, due to the advantages described in Table III, there is the possibility of repetition of the imaging exam when not well performed, without any biological damage to the patient, because this type of exam does not use ionizing radiation.

The heterogeneity of the masseter thickness reported in the literature is probably due to differences in sample selection, lack of standardization in the methodology, such as variation of the position and pressure of the transducer, the use of different ultrasound equipment and measurements performed by different observers (Oliveira *et al*.). Even so, it is possible accurately to determine the thickness of the mandibular elevator muscle following a specific protocol, carried out by an experienced appraiser, to guarantee the acquisition of values with the smallest possible error, through of the fulfillment of the defined protocol, with maximum necessary calibration (Satiroglu *et al*.).

According to other research, the application of a water-based gel is essential to reduce the noise of the sonogram and avoid the application of excessive pressure (Kiliaridis & Kälebo). The perpendicular positioning of the transducer is crucial for the correct ecographic visualization, if positioned obliquely; it leads to morphological distortion with consequent measurement error (Kiliaridis & Kälebo; Emshof *et al*.; Reis Durão *et al*.).

The linear measurements obtained in this research guarantee an estimate of the normality values for women and men. They are in accordance with the values described in the literature, showing that the menare thicker than women (Kiliaridis & Kälebo; Bakke *et al*.; Ariji *et al*., 2004; Satirogˇlu *et al*.; Georgiakaki *et al*.; Palinkas *et al*.; Reis Durão *et al*.). The discrepancy in values between males and females is due to the genetic variation found between the different genders (Palinkas *et al*.). The muscular composition is different between men and women, with a higher percentage of type II fibers in males. These muscle fibers present a more glycolytic phenotype, with a larger diameter and a predominance of anaerobic energy metabolism. The muscle made up of this type of fiber has a higher contraction speed and maximum tension compared to the female muscle, which is predominantly made up of type I muscle fibers (Lexell *et al*., 1988; Ciciliot *et al*., 2013).

Studies have shown that during growth there is a tendency to gain muscle mass, instead of what happens with aging, in which there is a natural degenerative process, tendently responsible for the loss of muscle mass (Frugone *et al*.; Oliveira *et al*.; Yamaguchi *et al*., 2018). However, the correlation between the age of the individual and the thickness of the MM is still controversial, in this study the average age of the individuals was 23.5 ± 2.3 years, with a limitation, as it is a relatively age group of young adults. Because, according to data in the literature, the reduction in muscle mass of the masseter is considered stronger in individuals affected by muscle inactivity, instead of aging (Yamaguchi *et al*.). So, this is one of the main causes of reduced muscle mass in older individuals. Muscle fiber atrophy associated with disuse is known to involve predominantly type I fibers (Ciciliot *et al*.), while aging involves type II fibers (Lexell *et al*.). Therefore, it is possible to increase the thickness of the MM through occlusal rehabilitation in healthy elderly patients, regardless of their age (Yamaguchi *et al*.).

Although some studies suggest a relationship between the age factor and the thickness of the MM (Raadsheer *et al*.) older and male individuals have a thicker muscle (Raadsheer *et al*.); other authors did not find the same relationship when selecting a sample of a reduced age group (Kiliaridis & Kälebo). On the other hand, all individuals observed present apparent occlusal stability, with no teeth other than premolars (for orthodontic reasons) or third molars being absent, as well as not having dental implants, especially removable. The balanced distribution of occlusal forces is essential for the symmetrical activation of the masticatory muscles, particularly MM, ensuring less effort and greater strength during function (Rastogi *et al*., 2015). Occlusal stability is translated as a functional variable, since it expresses the positive relationship between occlusal contacts and muscle thickness (Frugone *et al*.). Individuals with unilateral posterior crossbite have reduced MM thickness, ipsilateral to the crossbite (Kiliaridis *et al*.). Otherwise, premature occlusal contacts affect the masticatory function, generating an imbalance of the masticatory system, with suppression of oxygenation and accumulation of metabolic products, with consequent muscle pain (Lauriti *et al*., 2014).

Regarding "nutritional habits", studies point to the possibility of this variable influencing the morphology of MM (Hichijo *et al*., 2014; Oliveira *et al*.), showing that regions where the diet is fattier or essentially composed of meat, individuals tend to have greater masticatory strength and, consequently, an increase in the thickness of the masticatory muscles (Oliveira *et al*.). On the other hand, authors argue that the influence of diet on the stomatognathic system is visible only during growth (Hichijo *et al*.). Through the questionnaires it was possible to verify that 26.7 % of individuals, on average, consume meat seven times a week, this may indicate that there is a relationship between MM thickness, bite force, facial morphology, presence occlusal factors (Bakke *et al*.) and chewing, as in this research the sample consisted of young adult individuals (mean age and 23.5 ± 2.3 years). Since the hypothesis of the relevance of the individual's diet, mainly the consumption of protein, can be based on the anomalous relationship of that of muscular form and function (Oliveira *et al*.). The deterioration of masticatory efficiency restricts the intake of proteins and carbohydrates, consequently, nutrition (Saarela *et al*., 2016), thus increasing the risk of malnutrition.

Nevertheless, the "lifestyle" can affect the dimensions of the MM. Dental clenching requires the activation of the jaw elevator muscles, including MM, particularly in situations that require concentration, emotional peaks, and physical activity. When prolonged, joint and muscle overload occurs, leading to pain and functional impairment (Reynolds *et al*., 2016). Investigations show a correlation between occlusion and posture, demonstrating that the myocentric position of the mandible is favorable to postural balance (Fujino *et al*., 2010). Thus, sedentary lifestyle is also one of the etiological factors for poor posture, with significant consequences for all components of the musculoskeletal structure, including the craniofacial complex.

Regular physical exercise induces changes in contractile function and muscle fiber morphology (Bex *et al*., 2017). When the muscle contracts against some resistance, protein synthesis is stimulated, forming cracks in the muscle fibers (Camera *et al*., 2017). After recovery from exercise, new proteins are produced and muscle fibers recover, becoming denser and causing muscle hypertrophy (Locatelli *et al*., 2017). In addition to age and genetics, other factors such as unhealthy diet, limited physical activity, and the occurrence of chronic diseases also contribute to the loss of muscle mass and decreased muscle strength (Goodpaster *et* *al*., 2006). Other authors have found a positive correlation between masticatory ability and physical activity in elderly patients. In a study that showed the efficiency of MMs related to physical fitness in these patients (Gaszynska *et al*., 2014).

The following limitation of this study must be mentioned: the reduced sample within the groups (female and male) made it difficult to analyze a precise relationship between lifestyle and diet related to MM thickness. On the other hand, the findings reported here expand directions for future research on the influence of the muscle activity of masticatory muscles, the interconnection among eating habits, the sedentary lifestyle, and the characteristics of dental occlusion.

In this study, individuals with significant changes in the craniofacial complex were excluded, especially the cases with signs and / or symptoms of temporomandibular disorders. The presence of this pathological condition is responsible for a change in the stomatognathic complex, generating an imbalance in the temporomandibular joint, with consequent intramuscular modulation (Aldemir *et al*.; Ariji *et al*., 2004; Georgiakaki *et al*.). Muscle pain and fatigue are responsible for restricting function, preventing the maximum voluntary contraction of the MM as well as its relaxation, leading to a sub-measurement of thickness values ??(Ariji *et al*, 2010.).

The location of the masseter was determined by its palpation in relaxation and contraction (Bertram *et al*., 2003), followed by placement of the transducer for confirmation, asking the patient to repeat the alternate muscle movement, to distinguish between the skin and the muscular fascia (Close *et al*.). The MM is identifiable as a homogeneous structure, comprised between the skin and the mandible branch, easily perceived (Kiliaridis & Kälebo).

The values obtained in this study, female with 11.75 ± 1.14 mm in contraction and 10.19 ± 1.04 mm in relaxation and male with 13.95 ± 1.62 mm in contraction and 11. 64 ± 1.68 mm in relaxation, are similar to those found by other authors (female with 11.55 ± 0.5 mm in contraction and 8.8 ± 0.3 mm at rest and male with 14.2 mm ± 0.4 mm in contraction and 10.95 ± 0.3 mm at rest (Palinkas *et al*.), showing ultrasound as a precise method, but with reduced reproducibility in the relaxed mode, which can be explained by the fact that the muscle be highly susceptible to pressure applied by the transducer (Bertram *et al*.; Emshof *et al*.; Kiliaridis & Kälebo; Oliveira *et al*.), in addition to the variability of the resting position performed by individuals (Emshof *et al*.). On the other hand, the measurement in maximum intercuspation requires the significant collaboration of the patient to maintain occlusal contacts with maximum contraction. the MM (Kiliaridis & Kälebo), which translates into the maximum muscle thickness (Palinkas *et al*.).

Despite the data obtained, further studies are needed to support the evidence of the influence of diet on muscle development, while still allowing the distinction between different dietary regimes and the development phase in which the effect becomes more evident. In addition, the facial type will be considered in future studies, to enhance the diagnosis of masseter muscle pathology.

From the data obtained in this study and correlating them with the literature, it was possible to prove the reliability of the ultrasound in determining the thickness of the MM since a standardized measurement protocol was used. However, the values obtained through this method may vary according to ethnicity, different age groups, gender, and body mass index of individuals in a population.

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RESUMEN: El objetivo de este estudio fue determinar los valores medios del grosor del músculo masetero, en reposo y contracción, en hombres y mujeres sanos. Inicialmente se distribuyeron 102 cuestionarios entre los estudiantes. Se seleccionaron 30 pacientes, 15 hombres y 15 mujeres, según los criterios de inclusión, excluyendo a todos los individuos con síntomas de disfunción temporo-mandibular o síndromes con alteraciones craneofaciales. El grosor del músculo masetero se determinó de forma bilateral, en reposo y contracción. Como punto de referencia para la medición del espesor (plano axial), la prolongación posterior de la comisura labial. Los valores de normalidad del músculo masetero en las mujeres fue de 11,75 ± 1,14 mm en contracción y 10,19 ± 1,04 mm en relajación; y en los varones 13,95 ± 1,62 mm en contracción y 11,64 mm ± 1,68 mm en relajación. Los resultados obtenidos coinciden con los descritos en la literatura, señalando la precisión y reproducibilidad del ultrasonido si se aplica un protocolo, principalmente dirigido a controlar la presión aplicada por el transductor.

PALABRAS CLAVE: músculo masetero, ecografía, espesor, morfología craneofacial.

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