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Ultrason Imaging 2013 35: 307
DOI: 10.1177/0161734613502468

Article

Ultrasonic Imaging
35(4) 307–317

DOI: 10.1177/0161734613502468
ultrasonicimaging.sagepub.com

Three-Dimensional Evaluation of Masseter Muscle in Different Vertical Facial Patterns: A Cross-Sectional Study in Growing Children

Roberta Lione[1], Lorenzo Franchi[2,3], Andrea Noviello[1], Patrizio Bollero[1], Ezio Fanucci[1], and Paola Cozza[1]

Abstract

The aim of the present study was to analyze the anatomical three-dimensional (3D) characteristics of masseter muscle in growing subjects with different vertical patterns by using an ultrasound (US) method. The sample comprised 60 prepuberal subjects (33 males, 27 females) with a mean age of 11.5 ± 1.6 years with late mixed or permanent dentition and Class I molar and skeletal relationship. For each subject, a lateral cephalogram was required, and according to the mandibular plane angle (Frankfort horizontal plane/mandibular plane angle [FMA]), the subjects were divided into three groups of different underlying vertical facial patterns: brachyfacial: FMA < 22°, mesofacial: 22° ≤ FMA ≤ 28°, and dolichofacial: FMA > 28°. For each subject, an US scan was carried out to analyze the width, the thickness, the cross-sectional area, and the volume of the masseter muscle. Mean differences in measurements between vertical facial subgroups were contrasted by means of analysis of variance (ANOVA) with Tukey's post hoc tests ($p < 0.05$). Measurements of the whole masseter in dolichofacial patients were significantly smaller when compared with brachyfacial and mesofacial individuals during relaxation and contraction. The volume of the masseter decreased significantly by 10% going from the brachyfacial group to the mesofacial group and from the mesofacial group to the dolichofacial group with no difference between the left and the right sides. A significant negative correlation was found between the US measurements and the divergency (FMA°). Ultrasound is a technique indicated in children for evaluating muscles of mastication in vivo. Growing patients with a dolichofacial vertical pattern present with a reduced dimension of the masseter when compared with brachyfacial and mesofacial subjects.

Keywords

masseter muscle, 3D ultrasonography, growing subjects, vertical facial features, craniofacial muscles

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Introduction

It is well recognized that there are different underlying patterns in the vertical dimension of the face,[1] and various studies[2-6] have shown an association between masticatory muscles and vertical craniofacial morphology.

The force developed by a muscle is based substantially on its anatomic structure, and the masseter muscle, in particular, represents the functional capacity of the masticatory apparatus.[7,8] Radsheer et al.[9,10] and Bakke et al.[11] showed that ultrasonography could be used successfully to demonstrate variations in the size of masseter muscle because the superficial position of the masseter allows easy access for application of quantitative measurements.[9-12] Many investigators have found a significant correlation between facial dimension and the ultrasound (US) thickness of the masseter in adults reporting that the masseter muscle size is greater in short-faced subjects than in long-faced subjects.[4,11,13-16] As reported by Kiliaridis,[6] the masticatory muscles are able to influence the craniofacial growth in the presence of increased muscle activity, but not necessarily when the activity is reduced as in long-faced subjects.[6]

However, very little has been published about the development of the muscles of mastication and their relationship with facial morphology during growth. It is generally accepted that a close correlation exists between the size of the masseter muscle and the craniofacial form, but it is still unclear the influence of the mandibular muscles on the vertical development of the dentofacial complex in prepuberal individuals. In particular, Kitai et al.[17] suggested that, although genotype is important, mechanical stress brought by the volume of certain masticatory muscles, such as the masseter muscle, might influence the development of adjacent craniofacial skeletal regions. In the literature, controversy exists as to whether genetically determined facial morphology determines the strength of the masticatory muscle or vice versa. Radsheer et al.[9] and Chan et al.[3] reported a significant positive relation between the masseter thickness and the intergonial and bizygomatic width. In contrast, Charalampidou et al.[7] pointed out that during contraction the association between vertical craniofacial morphology, masseter thickness, and mechanical advantage was generally moderate in children.

The aim of the present study was to analyze the three-dimensional (3D) anatomical characteristics and the volume of masseter muscle in growing subjects with different vertical patterns by using a noninvasive ultrasonographic method of investigation. Volume-rendering 3D software allowed for the manipulation of the original data to display selectively specific features of the masseter muscle and to distinguish accurately differences in patients with different facial divergency.

Materials and Method

From a study sample of 275 consecutive subjects who sought orthodontic treatment at the Department of Orthodontics of the, University of Rome Tor Vergata, observed from June 2011 to December 2012, 60 children (33 males, 27 females) with a mean age of 11.5 ± 1.6 years (range = 8.2-13.7) were selected according to the following inclusion criteria: prepuberal stage of development (CS1-CS3 in cervical vertebral maturation[18]), late mixed or permanent dentition, and Class I molar and skeletal relationship. Exclusion criteria included absence of first molars, skeletal or functional asymmetries, temporomandibular joint diseases, genetic diseases, and previous orthodontic treatment.

This project was approved by the Ethical Committee at the University of Rome Tor Vergata, and informed consent was obtained from parents.

For each subject, a lateral cephalogram was required for routine diagnostic purposes.

According to the mandibular plane angle (Frankfort horizontal plane/mandibular plane angle [FMA]), the subjects were divided into three groups of different underlying vertical facial patterns (VFPs)[19]:

1. Brachyfacial (20 subjects: 9 males, 11 females): FMA < 22°;
2. Mesofacial (20 subjects: 11 males, 9 females): 22° ≤ FMA ≤ 28°;
3. Dolichofacial (20 subjects: 13 males, 7 females): FMA >28°.

For each subject, an US scan was carried out at the Department of Radiology of the University of Rome Tor Vergata. All examinations were performed by a single trained radiographer at the same real-time scanner (xSTREAM iU22, Philips Medical System, Amsterdam, The Netherlands).

The scans were performed bilaterally during relaxation and contraction to verify whether differences of morphological characteristics among the three groups were present or more evident at rest or during the function. The trials were conducted in a darkened room with the subjects seated upright with the Frankfort Plane parallel to the floor. A 20-mm thick standoff was placed directly over the masseter region. In the relaxed state, the patients were instructed not only to relax but also to maintain slight interocclusal contacts to avoid muscle stretching as a result of mouth opening. In the contracted state, the participants were asked to clench maximally in centric occlusion.

To analyze the masseter muscle, a broadband linear array volumetric transducer was used (VL13-5 Volume Linear Array, Philips xMATRIX Array Technology, Philips Medical System, Amsterdam, The Netherlands). To allow the best visualization of the masseter muscle, this linear array was used with 7 MHz operating frequency,[13] fine pitch, 192 elements, and high-resolution linear array.

According to the technique by Weijs and Hillen[20] and Van Sprosen et al.,[21] the probe was visually oriented at 30° to the Frankfort plane, perpendicular to the ramus of the mandible to register the scan planes at right angles to the long axis of the muscle and to avoid bias in obtaining the real size of the masseter muscle.

The images were reconstructed using Philips QLAB 3DQ GI (General Imaging 3D Quantification, Philips Medical System, Amsterdam, The Netherlands) directly on the machine or on a PC workstation. The volumetric transducer allowed to acquire 3D set of data in the field of view of the probe instantly and simultaneously. Through the QLAB software, the radiographer analyzed scans on the three planes of space, particularly longitudinal and cross sections of the muscle. Muscle length was computed by positioning an electronic cursor at the top and at the bottom of longitudinal slices, parallel with the long axis of the muscle image.[13] After the scanning of the masseter, the software automatically divided the longitudinal sweep of the muscle into eight equal parts by seven cross-sectional slices of 1 mm thickness. The operator predefined the number of slices. Evaluating the number of slice necessary, it was observed that seven slices allowed volumetric measurements of the masseter with no significant differences with respect to a greater number of slices and at the same time made the post-processing not so much time-consuming. The three central slices were selected to measure on each slice width, thickness, and cross-sectional area (CSA) by outlining manually the boundaries of the masseter with an electronic cursor (Figure 1). Width was measured as the linear distance between the most anterior and the most posterior points of the muscle cross section. Thickness was measured as the linear distance between the most superficial and the deepest points of the muscle cross section. CSA was calculated by using electronic cursors to manually outline the muscle boundaries.

For each of these parameters, the main value obtained from measurements taken on the three central slices was used for statistical analysis. Finally, after manually outlining the boundaries on the seven slices, the volumetric image of the masseter was automatically generated (Figure 2). When reconstructing the complete muscle image, the software interpolated the muscle contour in between seven slices. Each slice, therefore, represented a muscle volume equal to the CSA multiplied by the slice interval. The complete array of delineated slices representing the 3D muscle

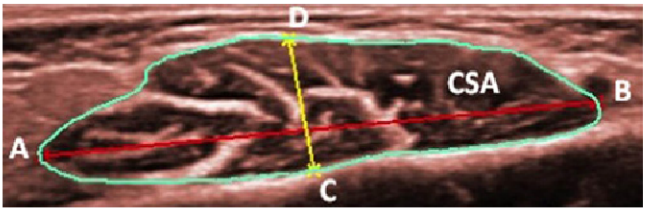

Figure 1. Cross-sectional US image of masseter muscle.
Note. AB: width measured as the linear distance between points A and B, corresponding to the most anterior and the most posterior points of the muscle cross section; CD: thickness measured as the linear distance between points C and D, corresponding to the most superficial and the deepest points of the muscle cross section; CSA: cross-sectional area calculated by using electronic cursors to manually outline the muscle boundaries. US = ultrasound.

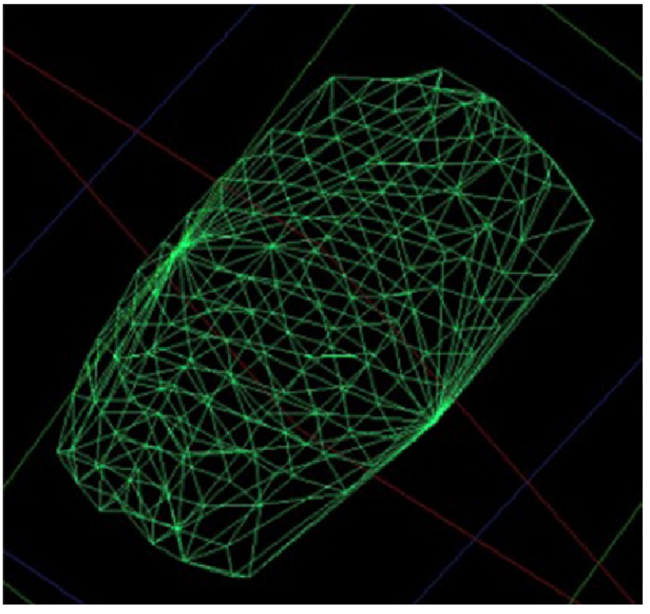

Figure 2. 3D volume rendering of the masseter muscle.
Note. 3D = three dimension.

image was then displayed. It could be rotated and viewed in all three planes of spaces, and it allowed to select individual slices or sections of the image.

Cephalometric Measurements

A lateral cephalometric radiograph was taken at the same time of US scan for each subject in centric occlusion. Conventional skeletal landmarks were traced by one Author (R.L.) and five standard angles were measured (Figure 3): SNA (point A to Sella-Nasion), SNB (point B to

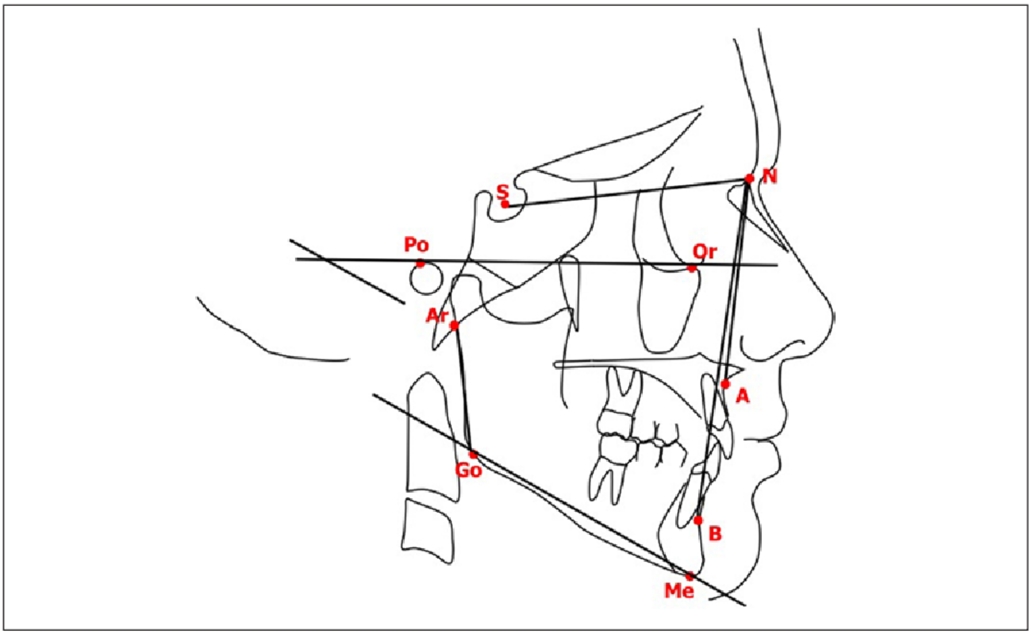

Figure 3. Cephalometric measurements: SNA (point A to Sella-Nasion), SNB (point B to Sella-Nasion), ANB (point A to Nasion to point B), FMA (Frankfort horizontal plane/mandibular plane angle), and gonial angle (posterior border of the mandible/inferior border of the mandible).

Sella-Nasion), ANB (point A to Nasion to point B), FMA (Frankfort horizontal plane/mandibular plane angle), and gonial angle (posterior border of the mandible/inferior border of the mandible).

US Measurements

All measurements and reconstructed images were performed on a computer workstation after the scans taken by the same examiner to evaluate:

- thickness-relaxed (TR; cm): mean value of masseter thickness on the right and left side during relaxation;
- thickness-clenched (TC; cm): mean value of masseter thickness on the right and left side during contraction;
- width-relaxed (WR; cm): mean value of masseter width on the right and left side during relaxation;
- width-clenched (WC; cm): mean value of masseter width on the right and left side during contraction;
- CSA-relaxed (CSAR; cm^2): mean value of masseter CSA on the right and left side during relaxation;
- CSA-clenched (CSAC; cm^2): mean value of masseter CSA on the right and left side during contraction;
- volume-relaxed (VR; cm^3): mean value of masseter volume on the right and left side during relaxation;
- volume-clenched (VC; cm^3): mean value of masseter volume on the right and left side during contraction.

Statistical Analysis

To determine the reliability of the cephalometric method, all radiographs were retraced and remeasured by the same examiner (A.N.) after an interval of approximately two weeks. The US measurements were automatically provided by the software after outlining manually the boundaries of the masseter with an electronic cursor. Double recording on 20 subjects were performed to evaluate the method error for the US measurements. The time interval between first and second recordings was four weeks. A paired *t*-test was used to compare the two cephalometric and US measurements (systematic error). The magnitude of the random error was calculated by using the Dahlberg's formula.[22] Exploratory statistics revealed that all variables were normally distributed (Kolmogorov–Smirnov test) with equality of variances (Levene's test). Mean differences in measurements between vertical facial subgroups were contrasted by means of analysis of variance (ANOVA) with Tukey's post hoc tests (SigmaStat 3.5, Systat software, Point Richmond, Richmond, CA, USA). An analysis of correlations between US measurements and facial divergency (FMA°) was performed by using the Pearson correlation coefficients. The power of the study for the ANOVA test was calculated for an effect size of 1 for the variable Volume-relaxed (VR),[13] an alpha level of 0.05, and sample size of 20. The power was 0.80. All statistical computations were performed with specific software (SigmaStat 3.5, Systat Software, Point Richmond, Richmond, CA, USA).

Results

No systematic error was found between the repeated cephalometric and US measurements. The method errors and coefficients of reliability[22] for the angular measurements ranged from 0.03° for ANB to 0.91° for gonial angle and from 0.948 for ANB to 0.999 for FMA, respectively. The error for masseter measurements did not exceed for thickness, 0.01 cm in relaxation and 0.02 cm in contraction; for width, 0.05 in relaxation and 0.04 in contraction; for CSA, 0.03 cm^2 in relaxation and 0.05 cm^2 in contraction; and for volume, 0.08 cm^3 in relaxation and 0.09 cm^3 in contraction. Descriptive statistics for cephalometric measurements, for US measurements, and the results of statistical analysis classified according to VFPs are listed in Table 1. No significant differences for sagittal measurements between subjects with different vertical patterns (SNA, SNB, and ANB) were found, while the FMA angle and the gonial angle increased significantly from the brachyfacial subjects to dolichofacial subjects. The masseter muscle increased its thickness, CSA, and volume during contraction regardless of the type of VFP. Dolichofacial subjects demonstrated significantly smaller values of thickness (TR: 0.6 cm, TC: 0.8 cm), width (WR: 4.0 cm, WC: 3.7 cm), and CSA (CSAR: 2.1 cm^2, CSAC: 2.4 cm^2) when compared with brachyfacial (TR: 0.9 cm, TC: 1.2 cm, WR: 4.6 cm, WC: 4.2 cm, CSAR: 3.1 cm^2, and CSAC: 3.6 cm^2) and mesofacial individuals (TR: 0.8 cm, TC: 1.0 cm, WR: 4.5 cm, WC: 4.0 cm, CSAR: 2.6 cm^2, and CSAC: 3.3 cm^2). No significant differences were found between brachyfacial (TR: 0.9 cm, WR: 4.6 cm, WC: 4.2 cm) and mesofacial (TR: 0.8 cm, WR: 4.5 cm, WC: 4.0 cm) subjects for masseter thickness in relaxed state and for masseter width during relaxation and contraction (Figure 4). The volume of the masseter muscle decreased significantly by 10% going from the brachyfacial group (VR: 14.9 cm^3, VC: 16.7 cm^3) to the mesofacial group (VR: 13.5 cm^3, VC: 14.9 cm^3) and from the mesofacial group to the dolichofacial group (VR: 11.8 cm^3, VC: 12.7 cm^3) during relaxation and contraction. When subjects were categorized according to the different VFPs, the US measurements showed a significant tendency ($p < 0.001$) to be inversely related to the facial divergency (FMA°) during relaxation and contraction (Table 2).

Table 1. Descriptive Statistics and Statistical Analysis for US and Craniofacial Variables (Classified According to the Vertical Facial Patterns).

	Brachyfacial (FMA < 22) (20 Subjects: 9 Males, 11 Females)		Mesofacial (22 ≤ FMA ≤ 28) (20 Subjects: 11 Males, 9 Females)		Dolichofacial (FMA > 28) (20 Subjects: 13 Males, 7 Females)		Brachyfacial vs. Mesofacial	Mesofacial vs. Dolichofacial	Brachyfacial vs. Dolichofacial
	M	*SD*	*M*	*SD*	*M*	*SD*	*p*	*p*	*p*
Age (years)	11.8	1.4	11.6	1.7	11.0	1.5			
Muscular measurements									
TR (cm)	0.9	0.1	0.8	0.1	0.6	0.1	NS	***	**
TC (cm)	1.2	0.1	1.0	0.1	0.8	0.1	**	***	**
WR (cm)	4.6	0.4	4.5	0.4	4.0	0.4	NS	**	***
WC (cm)	4.2	0.3	4.0	0.4	3.7	0.3	NS	**	***
CSAR (cm^2)	3.1	0.4	2.6	0.5	2.1	0.4	**	***	***
CSAC (cm^2)	3.6	0.4	3.3	0.6	2.4	0.4	*	***	***
VR (cm^3)	14.9	0.9	13.5	1.2	11.8	1.0	***	***	***
VC (cm^3)	16.7	1.1	14.9	1.4	12.7	1.1	***	***	***
Cephalometric measurements (°)									
SNA	81.7	3.0	81.3	3.3	79.4	3.4	NS	NS	NS
SNB	78.8	2.9	78.3	3.2	76.7	3.4	NS	NS	NS
ANB	2.9	1.4	3.1	1.3	2.7	1.5	NS	NS	NS
FMA	19.3	2.1	24.8	1.8	30.8	2.9	***	***	***
Gonial angle	124.6	2.1	130.0	2.9	135.1	3.1	***	***	***

Note. US = ultrasound; TR = thickness-relaxed; NS = not significant; TC = thickness-clenched; WR = width-relaxed; WC = width-clenched; CSAR = cross-sectional-area-relaxed; CSAC = cross-sectional-area-clenched; VR = volume-relaxed; VC = volume-clenched; SNA = point A to Sella-Nasion; SNB = point B to Sella-Nasion; ANB = point A to Nasion to point B.
*$p < 0.05$. **$p < 0.01$. ***$p < 0.001$.

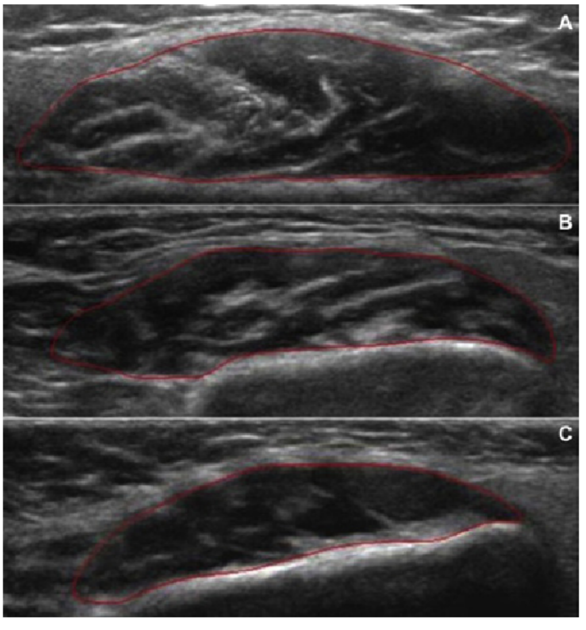

Figure 4. Cross-sectional US image of the masseter muscle in a brachyfacial subject (A), mesofacial subject (B), and dolichofacial subject (C) during relaxation.
Note. US = ultrasound.

Discussion

The aim of the present study was to analyze the anatomical characteristics of masseter muscle in growing subjects with different underlying VFPs by means of a noninvasive ultrasonographic method with no cumulative biological effects on living tissue.[10] These factors render ultrasonography an appropriate technique for precision analysis of muscle shape especially for large-scale studies in children.[23] A variety of techniques[4,9-16,24,25] have been used in the literature for the evaluation of masseter muscle, but the technique applied in the present study has been reported by most of the authors.[24] Representative indices of the size of the masseter are its thickness, width, CSA, and volume.[26] By analyzing skeletal muscle in cadaver material, it has been shown[26] that single slice variables (such as thickness, width, and CSA) are more prone to measurement errors than muscle volume. In the present study, this effect was small as the width, thickness, and CSA were defined as the mean value obtained from the three central slices, also preventing variance of masseter dimension that may result from the location of the measured point.

As seen from data in Table 1, the masseter muscle increased its thickness, CSA, and volume during contraction regardless of the type of VFP. This outcome is in agreement with several studies that evaluated outer shape changes of adult masseter with contraction.[4,10,24] A previous study on US measurements of masseter muscle in normal and open-bite subjects showed no significant difference in thickness between the two groups.[27] This difference is probably due to the different technique applied in the study of Neeta and Mushni[27] and to very small sample size (20 subjects) with a large range of age (8-17 years). In the present study, the dolichofacial subjects demonstrated significantly smaller values for masseter thickness, width, and CSA with respect to brachyfacial and mesofacial subjects indicating that individuals with small and thin masseters have a long face. No differences were pointed out for thickness in relaxed state and width when comparing brachyfacial and mesofacial subjects. Several studies[28-31] pointed out the possibility of training the masticatory muscle in growing subjects with high angle mandibular pattern by introducing an acrylic splint in the posterior region of the arch. The orthodontic effects of increasing maximum bite force and volume of the masseter could lead to a clockwise rotation of the mandible with the final effects of reducing the vertical dimension in growing subjects with dolichofacial patterns. The smaller dimension of masseter muscle in growing subjects with high angle mandibular pattern is consistent with previous reports.[9,11,13,15,32] Van Sprosen et al.[5] reported values of the CSA greater than the values of the present study pointing out that long-faced adults had significantly smaller masseters than normal subjects. Direct comparisons between these studies were, however, difficult to make. The differences can be attributed to variation in ethnicity, age of the sample group, method of analysis, and choice of the scanning level.

Finally, to our knowledge, this is the first attempt to evaluate the volume of the masseter muscle in a notably large homogeneous sample with regard to previous reports that used US imaging. In the present study, the young age of the study sample as well as the use of a 20-mm thick standoff allowed visualization of the masseter muscle from the inferior border of the zygomatic arch to the gonial angle of the mandible. The volume values in subjects with different vertical patterns reported in Table 1 indicated that as the masseter became with a greater volume, mandibular inclinations (FMA) tended to become significantly smaller. In particular, the volume of the masseter decreased significantly by 10% going from the brachyfacial group to the mesofacial group and from the mesofacial group to the dolichofacial group during relaxation and contraction with no difference between the left and the right sides. Significant negative correlations between US measurements and facial divergency (FMA°) were found suggesting that a larger masseter corresponds to a small gonial angle with a flat mandibular plane in subjects with a short-face tendency (Table 2). These results are in agreement with the study of Benington et al.,[13]

Table 2. Correlations between Facial Divergency (FMA°) and Muscular US Measurements.

Muscular Measurements	FMA°
TR	
Pearson correlation	−0.544
Significance (two-tailed)	0.000
TC	
Pearson correlation	−0.537
Significance (two-tailed)	0.000
WR	
Pearson correlation	−0.660
Significance (two-tailed)	0.000
WC	
Pearson correlation	−0.713
Significance (two-tailed)	0.000
CSAR	
Pearson correlation	−0.680
Significance (two-tailed)	0.000
CSAC	
Pearson correlation	−0.703
Significance (two-tailed)	0.000
VR	
Pearson correlation	−0.749
Significance (two-tailed)	0.000
VC	
Pearson correlation	−0.760
Significance (two-tailed)	0.000

Note. TR = thickness-relaxed; TC = thickness-clenched; WR = width-relaxed; WC = width-clenched; CSA = cross-sectional area; CSAR = cross-sectional-area-relaxed; CSAC = cross-sectional-area-clenched; VR = volume-relaxed; VC = volume-clenched.

Boom et al.,[2] and Gionhaku and Lowe[33] who reported a correlation between the volume and the vertical dimension in adults, respectively, by means of US, magnetic resonance, and computed tomography. However, a relatively direct comparison can be made only with the study of Chan et al.[3] who analyzed a study sample of 20 subjects with the same mean age of 11.9 years by using computed tomography. These authors found similar values for the CSA and the volume of the masseter demonstrating the reliability of the US method applied in the present investigation.

Further investigations are necessary to assess which mandibular muscles may influence craniofacial morphology or vice versa and to evaluate the possibility of training mandibular muscle by orthodontic treatment in growing subjects.

Conclusion

- Ultrasound is a technique indicated for evaluating muscles of mastication in vivo in children, as it allows a two-dimensional (2D) and a 3D analysis of the whole masseter.
- The results showed significant differences in masseter muscle dimensions between different VFPs.
- Growing subjects with a dolichofacial vertical pattern presented with a reduced dimension of the whole masseter when compared with brachyfacial and mesofacial individuals.

- The volume of the masseter muscle decreased significantly by 10% going from the brachyfacial group to the mesofacial group and from the mesofacial group to the dolichofacial group during relaxation and contraction with no differences between the left and the right sides.
- A significant negative correlation was found between US measurements and facial divergency (FMA°) suggesting an influence of the masseter dimension on the vertical dimension in growing subjects.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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