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RESEARCH

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Quantitative analysis of masseter muscle by ultrasonography according to different occlusion types using Eichner classification in Turkish subpopulation

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Abstract

Background The aim of this study is to investigate the effects of different occlusion types based on the Eichner classification index on the quantitative features of the masseter muscle by using USG and to evaluate whether these features differ according to age and gender in Turkish subpopulation.

Methods The thickness and elasticity values of the masseter muscle were performed. Images were acquired bilaterally in the resting position and maximum intercuspidation. The significance level was set as $p = 0.05$.

Results Measurements of 120 people showed that the thickness of the right masseter muscle was lower in women compared to men, both when relaxed and when contracted. The thickness of the left masseter muscle was also lower in women when it was contracted. This difference is significant ($P < 0.01$). The thickness of the right and left masseter muscles at rest in individuals aged 55 and over was significantly less than those in the 18–35 age range ($P < 0.01$). The thicknesses of the right and left masseter muscles at rest were lower in individuals in the Eichner C3 category, while in the contracted state, they were lower in individuals in the B3 and B4 categories.

Conclusion Clinicians should consider these variations in demographic and dental status when designing dental and orthodontic interventions. Taking these factors into account can improve chewing ability and customize treatments better, which may lead to better results for patients of different backgrounds.

Trial registration Clinical trial number: Not applicable.

Keywords Eichner classification, Elasticity imaging techniques, Masseter muscle, Ultrasonography

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Background

The masseter muscle has an important structural and functional role in the stomatognathic system [1]. It is the largest elevator muscle of the jaw and makes the greatest contribution to jaw closure [2]. Age, dental health, salivary flow, temporomandibular joint (TMJ) disorder, and orofacial pain all influence masticatory function [3]. And the most common masticatory muscle disorder is TMD, which affects 34% of the population % (Asia—33%, South America—47%, North America—26%, Europe—29%) [4]. Especially in Turkish adults, the rate of those showing TMD symptoms is 69.8% [5].

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Number of functional teeth and bite force are considered key determinants of chewing function [6]. Although chewing is usually bilateral, 78% of patients prefer the area where the tooth contacts most during lateral shifting [7]. The most important factor affecting tooth contact during lateral shift is loss of function in the post-canine region [8].

The Eichner classification, an index system based on occlusion, is used to classify occlusal supports in the post-canine (premolar and molar) region [9]. According to the Eichner classification, occlusal power decreases more in the group with less occlusal support [3].The bite force in individuals aged 35–44 is 40% higher than in those aged 75 and over [10]. Because of tooth loss, the chewing muscles cannot work as strongly as in the full dentition period, but the main reason for the decrease in bite force with reduced workload is thought to be atrophy in the muscles that elevate the jaw. In addition, age progression increases muscular atrophy, too [11, 12]. The size of the masticatory muscles, especially the masseter and medial pterygoid muscles, gets smaller, and there is a negative relationship between them [13].

Tooth-loss-related and age-related muscle atrophy may be evaluated by using surface electromyography (sEMG) [14], magnetic resonance imaging (MRI), computed tomography (CT), and ultrasonography (USG) [15]. The USG is an imaging method that is simple, easily accessible, noninvasive, low-cost, lacks ionizing radiation, can be readily accepted by patients, and provides real-time imaging and follow-up [16, 17]. It is a preferred imaging method in the evaluation of the musculoskeletal system because of its practical and dynamic image [17]. For the assessment of muscle atrophy, the most frequently investigated parameter is the masseter muscle because of its superficial quadrate nature, easy measurement, and clinical importance [18]. There are studies on linear and cross-sectional volumetric measurements, including length, thickness, cross-sectional area, and volume [19], and elasticity with strain [20] or shear wave elastography (SWE) [21] of the masseter muscle with USG. The SWE, which is an objective and quantitative method, is used in the diagnosis and follow-up of pathological processes [22]. SWE is a real-time diagnostic imaging technique that provides quantitative information (in kPa or m/s) about tissue elasticity. SWE measures how fast shear waves move when local tissues are pushed by sound waves from probes. With SWE, it is aimed to reduce operator dependency and to provide reproducibility and quantitative evaluation [23, 24].

The aim of this study is to investigate the effects of different occlusion types based on the Eichner classification index on the quantitative features of the masseter muscle by using USG and to evaluate whether these features differ according to age and gender in Turkish subpopulation. We hypothesize that knowledge of various occlusion types, as well as age- and gender-related alterations in the masseter muscle, will enhance prosthetic and orthodontic treatment planning, offer initial insights into early pathological changes, and alleviate concerns for both patients and dentists regarding non-pathological variations associated with these factors.

Materials and methods

This cross-sectional study was approved by the Local Ethical Board (Approval Number and Date: 36290600/08/2023) and carried out in accordance with the guidelines of Helsinki Declaration [25]. This study adheres to the STROBE guidelines for reporting observational studies.

Power analysis was performed using G*Power 3.1.9.7 software (Kiel University, Kiel, Germany) with an F-test (ANOVA: Fixed effects, omnibus, one-way) for masseter muscle thickness, and the required sample size was determined as 90 based on an alpha level of 0.05, statistical power of 0.95, and an effect size of 0.426 [26].

Selection of individuals

Patients must be 18 years of age or older, have a full set of natural teeth including second molars, have not used prostheses for at least 3 months, have no history of orthodontic treatment, exhibit non-mobility, and show no marked facial asymmetry. On the other hand, the exclusion criteria include systemic and neuromuscular diseases, parafunctional habits, orofacial pain (including pain and tenderness in the masseter muscle and temporomandibular joint (TMJ)), developmental deformities or a history of surgery (such as facial trauma or resection) in the maxillofacial region, TMJ pathologies (major condylar changes visible on panoramic radiographs), use of medical drugs affecting the muscular system, and a history of radiotherapy and chemotherapy.

Individuals attending the faculty hospital for routine clinical assessments were inquired about their willingness to participate in the study, contingent upon their compliance with the inclusion-exclusion criteria. Consent, both written and verbal, was secured from participants who chose to engage in the study voluntarily.

Identification of gender and age categories

One hundred and twenty people engaged in the study. The participants, comprising 60 women and 60 men, were aged between 18 and 78 years. To achieve a fair age distribution among the groups, participants were categorized into four age brackets: 18–35, 36–54, and 55 and older.

Clinical and radiological examination

Bruxism is common and linked to a bigger masseter muscle [27]. Therefore, we tried to rule out bruxism during the clinical exam. Bruxism was systematically ruled out during the clinical examination.

For subjective assessment, participants were asked standardized questions related to bruxism, including:

- “Did you wake up biting your tongue?”
- “Have you noticed clenching or grinding your teeth while awake in the last six months?”
- “Have you experienced pain in the temporomandibular, temporal, or neck regions?“ [28].

Additionally, questions based on the American Academy of Sleep Medicine (AASM) Diagnostic Criteria [29] or sleep bruxism were included to improve diagnostic accuracy.

For objective assessment, a detailed intraoral and extraoral clinical examination was performed:

- Intraoral examination assessed signs such as tooth wear, broken restorations, frequent restoration fractures, bite marks on the tongue, cheeks, and lips, dry mouth, and excessive joint movement.
- Extraoral examination evaluated muscle stiffness, pain, limitations or deviations in mouth opening, facial asymmetry, square facial structure, and hypertrophy in the TMJ, masticatory, and trapezius muscles.

Orofacial pain, including pain and tenderness in the masseter muscle and TMJ, was assessed clinically according to the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) [30]. The evaluation included:

- Palpation of the masseter muscle and TMJ to identify tenderness and pain response.
- Assessment of jaw movements for any deviations, limitations, or discomfort during opening and closing.
- Patient-reported pain history, including questions about spontaneous pain, pain during function (chewing, speaking), and morning jaw stiffness.

Extraoral clinical examination evaluated muscle stiffness, pain, limitation, and deviation in mouth opening; facial asymmetry; square face; and muscle hypertrophy in the extraoral palpation of the TMJ, masticatory muscles, and trapezius muscle. In addition, as a result of intraoral (bite-wing, occlusal and periapical radiographs) and extraoral (panoramic radiography, cone beam computed tomography (CBCT) radiographic imaging taken during routine examinations depending on the indication, hypercementosis, periodontal damage, condylar degeneration (osteophytes, hyperplasia, atrophy etc.), facial asymmetry, alveolar ridge resorption, pulp necrosis and pulp stones were evaluated and the presence of bruxism and other exclusion criteria specified were completely excluded.

Categorization of participants according to the Eichner index classification

The Eichner index [9] is a dental metric that evaluates occlusal contacts between premolar and molar teeth. This indexing system comprises three primary groupings, each with subgroups. Eichner A collective comprises four support areas and consists of three subgroups: A1, A2, and A3. The A1 group exhibits no tooth loss, the A2 group presents with unilateral tooth loss, and the A3 group demonstrates bilateral tooth loss. The Eichner-B group has four subgroups. The B1 subgroup contains three support areas, the B2 subgroup has two support areas, and the B3 subgroup comprises one support area. In the B4 subgroup, contact is limited to the anterior region, with no support in the molar area. The Eichner C group, comprising three subgroups, is without a support area. The C1 subgroup denotes the existence of at least one tooth in both the maxilla and mandible; the C2 subgroup signifies the presence of at least one tooth in either the mandible or maxilla; and the C3 subgroup represents total edentulism.

Using the Eichner classification, they were split into 3 groups based on how many occlusal contacts they had—15 females and 15 males in each group: Eichner A, Eichner B, and Eichner C. For comparison, 30 healthy and fully dentate individuals were selected as the control group. Three observers classified the participants using the Eichner classification. Dentists with 6 years (Z.I.O.B) and 8 years (B.E.) of experience used articulation paper to find the contact points on the right and left sides. They took photos of the patient's occlusion in centric relation and agreed on the categorization. Then, (M.H.K.), who has 16 years of experience in oral diagnosis and radiology, reviewed their findings again. Interobserver agreement was calculated for Eichner categorization and was determined as 0.976.

This study aimed to investigate the effects of various occlusion types, as classified by the Eichner index, on the quantitative properties of the masseter muscle using ultrasound imaging. The Eichner index classification by itself does not provide clear information about where tooth loss occurs. For example, in the Eichner A2 classification, the missing teeth may occur on either the right or left side. This highlights the necessity for enhanced information in the Eichner classification concerning the objective assessment of the right and left masseter muscles. The control group, designated as the Eichner A1 class, comprises four occlusal support points, specifically two on the right and two on the left. Consequently, during the collection of patient data, both the Eichner index classification and the bilateral recording of right and left occlusal support points were documented as 0, 1, and 2.

Ultrasonography images dataset

The masseter muscles of each individual were examined with the ACUSON S 2000 (Siemens, Munich, Germany). Ultrasonographic examination of the thickness and elasticity values of the masseter muscle was performed.

Measurement of masseter thickness with a linear probe

18L6 HD probe with 12 MHz was used for analysis, and the B-mode imaging sequence for images. The imaging parameters included a focal range of 0.5–2.5 cm, an image depth of 5.5 cm, and a dynamic range of 65–90 dB. To determine the most voluminous superficial area of the masseter muscle, a horizontal line, 2 cm above the corpus and 1 cm in front of the ramus, was drawn parallel to the mandible corpus. A line was drawn 1 cm in front of the ramus, measuring at 1 cm intervals based on the front, back, and middle sections of the outer part (Fig. 1.a.) [31].

Water-based gel was applied between the probe and the skin in order to prevent air between them and to create a clear image. Very light pressure was applied to the probe so as not to compress the tissues. The probe was placed transversely on this line and the probe angle was adjusted perpendicular to the masseter muscle fibers

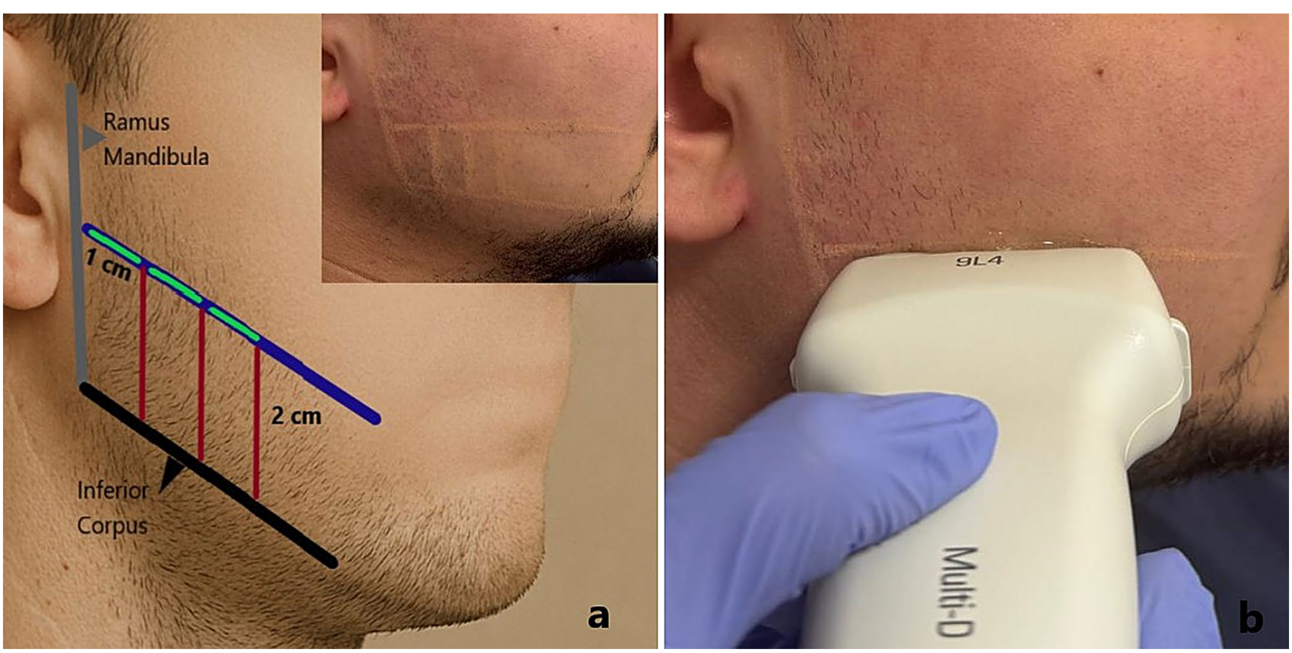

Fig. 1 **(a)** Illustration and clinical landmarks for the standardization of the USG measurements **(b)** Probe position during measurements of masseter muscle thickness and elasticity

and ramus. For this setting, the angle of the probe was changed until the image of the ramus appeared as a sharp, white, clear line on the screen (Fig. 1.b.). Images were obtained bilaterally in both the resting position and maximum intercuspidation (MIC) (Fig. 2). Muscle thickness was measured using ultrasonography in a resting position, with the individual at rest and without occlusal contact between the teeth. Participants were directed to seal their lips, swallow saliva, inhale deeply, and relax their jaw. Participants were directed to exert maximum bite force in centric occlusion to assess muscle thickness, with verbal encouragement given during the measurements. Measurements were conducted exclusively in edentulous patients while in the resting position.

A maxillofacial radiologist (Z.I.O.B) with six years of radiological expertise did the USG measurements to assure accuracy and consistency, adhering to the standards outlined below.

- The operator, who was right-handed, positioned herself to the patient's right and faced the patient throughout all measures.
- Ultrasound scans were conducted utilizing a substantial quantity of ordinary water-based gel between the participant's skin and the probe, ensuring no compression or pressure was applied to the tissues under examination.
- A pencil was employed to delineate the skin following palpation to precisely identify the most prominent region of the masseter muscle in a contracted position, ensuring technical uniformity across all patients.
- The operator stabilized the scanner hand by positioning the heel or little finger on the patient's head and neck to guarantee enough transducer contact with the skin and to mitigate involuntary movements.
- The operator refrained from resting his arm on the patient's chest to avoid interference with the measurements caused by breathing movements during the examinations.
- Scans and measurements were conducted again after 15 min. Three measurements were conducted. The mean of three measurements for the thickness of the right and left masseter muscles was documented individually for each participant [32].

Measurement of masseter elasticity with shear-wave elastography

Elastography tests on the masseter muscles were done using the 9L4 probe. These tests were carried out in the same spot and with the same settings after taking linear measurements. The kPa values, taken from different regions of interest (ROI) selected in the masseter muscle, were measured, and the mean values were recorded (Fig. 3). The measurement was repeated 3 times. The mean of three measurements for the elasticity of the right and left masseter muscles was documented individually for each participant.

Statistical analysis

Intraclass correlation coefficient (ICC) values were calculated to assess intra-observer reliability for muscle thickness and stiffness measurements. A total of 20 participants were included in the ICC analysis, ensuring robust reliability assessment. Statistical analysis of the obtained data was performed using Statistical Package for Social Science software for Windows (SPSS Statistics 22.0 software IBM Corp., NY, USA). The Kolmogorov-Smirnov test was used for conformity to normal

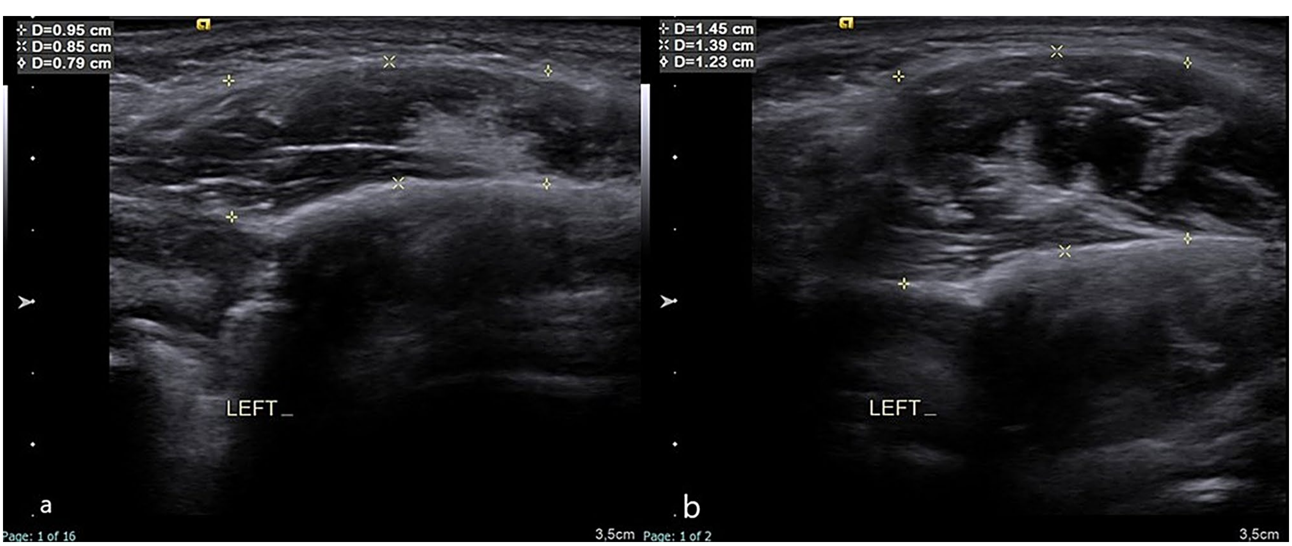

Fig. 2 Measurements of masseter muscle thickness with linear prob. **(a)** resting position; **(b)** maximum intercuspidation

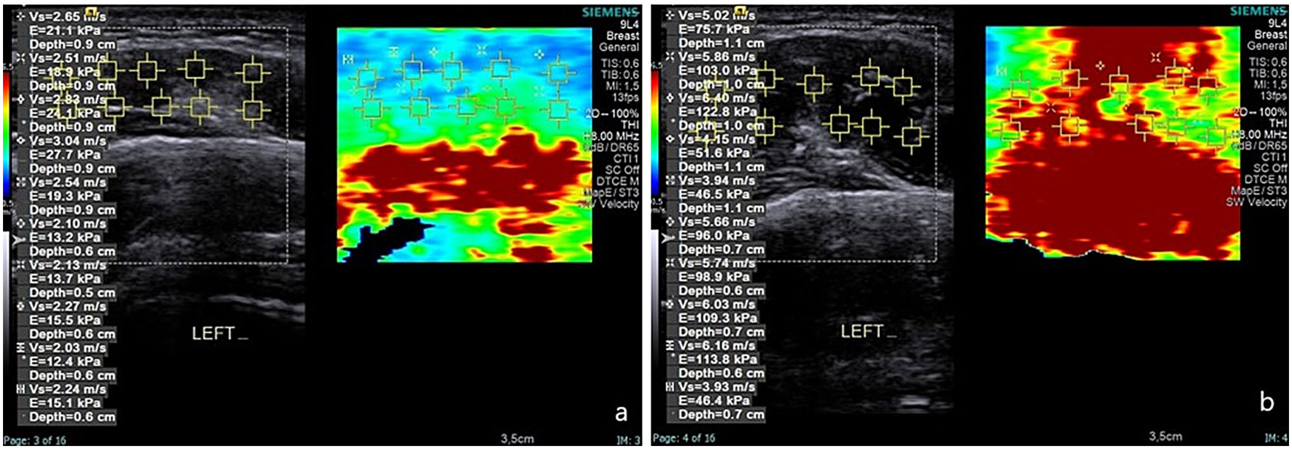

Fig. 3 Measurement of masseter muscle elasticity with shear-wave alestography. **a**. resting position; **b.** maximum intercuspidation

distribution for each measurement item. Chi-square, Mann Whitney U, and Kruskal Wallis tests were used. The significance level was set at $\alpha = 0.05$.

Effect sizes were reported alongside p-values to provide a measure of the magnitude of observed differences. For comparisons between two independent groups, Rank-Biserial Correlation (derived from the Mann-Whitney U test) was used as the effect size. For comparisons involving three or more independent groups, Epsilon Squared (ε^2) (derived from the Kruskal-Wallis test) was used to indicate the proportion of variance explained. Additionally, Cohen's d was reported for parametric group comparisons where appropriate.

Interpretation of effect sizes was based on conventional thresholds [33]:

- Cohen's d: small (< 0.50), moderate (0.50–0.80), large (≥ 0.80).
- Rank-Biserial Correlation: small (< 0.10), moderate (0.10–0.30), large (≥ 0.30).
- Epsilon Squared: small (< 0.01), moderate (0.01–0.06), large (≥ 0.06).

All effect sizes were presented with 95% confidence intervals (CIs) to indicate the range within which the true effect size is expected to lie.

Results

This research included a total of 120 individuals aged between 18 and 78 years (mean 46.94 ± 17.59 years), comprising 60 females and 60 males. The distribution of data according to age groups and gender is presented in Tables 1 and 2. The distribution of age groups by gender did not show any statistically significant difference ($p = 0.333$).

To assess intra-observer reliability, the thicknesses of the right and left masseter muscles were measured three times in both relaxed and contracted states, resulting in the following ICC values: 0.983 for the right masseter at rest, 0.996 for the right masseter during contraction, 0.995 for the left masseter at rest, and 0.993 for the left masseter during contraction.

The morphological characteristics of the masseter muscle based on gender are presented in Table 1 with mean and standard deviation values. According to measurements made on the right masseter muscle in both relaxed and contracted states and the left masseter muscle in a contracted state, thicknesses in females were significantly lower than those in males ($p < 0.01$, Rank-Biserial Correlation: 0.33, 95% CI: 0.09–0.57) (Table 3). It was found that people aged 55 and older had noticeably thinner masseter muscles at rest compared to those aged 18 to 35 ($p < 0.01$, Epsilon Squared: 0.17, 95% CI: 0.04–0.31) (Table 3). In people aged 55 and older, all measurements of the right and left masseter muscles were significantly lower than in younger age groups, except for the depth when the muscles were tightened ($p < 0.05$, Epsilon Squared: 0.12, 95% CI: 0.00–0.24) (Table 3).

Table 2 offers a detailed assessment of the number of occlusal teeth supports in terms of demographic characteristics and morphometric measurements. When looking at the number of teeth that support the bite, there was no meaningful difference between genders on either side ($p > 0.05$, Cohen's d: 0.45, 95% CI: 0.32–0.58) (Table 4). However, differences were found based on age groups ($p < 0.01$, Cohen's d: 0.60, 95% CI: 0.48–0.72) (Table 4). Particularly, a noticeable decrease in the number of occlusal tooth supports was observed in individuals aged 55 and over. Masseter muscle thickness and elasticity on the left and right sides were much lower in people without tooth contact. The contraction of the left masseter muscle specifically affected its thickness. The differences were significant ($p < 0.01$, Epsilon Squared: 0.22, 95% CI: 0.07–0.37) (Table 4).

Table 5 presents a comprehensive assessment of demographic characteristics and morphometric measurements

Table 1 Demographic distribution by gender and age categories with a detailed examination of masseter muscle morphometric measurements

	Gender			Age Groups				Total Sample
	Male	Female	*p* value	18–35 years	36–54 years	55 years and older	*p* value	
Total Sample (n/%)	60 (50.00%)	60 (50.00%)		38 (31.70%)	37 (30.80%)	45 (37.50%)		120 (100.00%)
Right Masseter Muscle Thickness (Relaxed) (cm)	1.02 ± 0.22	0.91 ± 0.17	0.004**	1.05 ± 0.18[a]	0.98 ± 0.17[a.b]	0.88 ± 0.21[b]	0.000**	0.96 ± 0.20
Right Masseter Muscle Thickness (Contracted) (cm)	1.36 ± 0.25	1.21 ± 0.2	0.001**	1.34 ± 0.19	1.25 ± 0.24	1.22 ± 0.29	0.094	1.28 ± 0.24
Left Masseter Muscle Thickness (Relaxed) (cm)	1.00 ± 0.2	0.96 ± 0.21	0.292	1.06 ± 0.21[a]	0.98 ± 0.18[a.b]	0.91 ± 0.19[b]	0.003**	0.98 ± 0.20
Left Masseter Muscle Thickness (Contracted)(cm)	1.33 ± 0.25	1.20 ± 0.19	0.002**	1.31 ± 0.22	1.23 ± 0.22	1.26 ± 0.25	0.397	1.27 ± 0.23
Right Masseter Muscle Elastography - Vs (Relaxed)	4.03 ± 0.89	4.08 ± 0.97	0.835	4.01 ± 0.96[a.b]	4.48 ± 0.82[a]	3.75 ± 0.87[b]	0.001**	4.05 ± 0.93
Right Masseter Muscle Elastography - E (Relaxed) E	53.03 ± 21.16	54.15 ± 22.81	0.875	52.68 ± 22.21[a.b]	63.83 ± 20.12[a]	45.93 ± 20.10[b]	0.001**	53.59 ± 21.92
Right Masseter Muscle Elastography - Depth (Relaxed)	1.19 ± 0.42	1.24 ± 0.35	0.415	1.28 ± 0.39[a]	1.36 ± 0.37[a]	1.05 ± 0.34[b]	0.000**	1.22 ± 0.39
Right Masseter Muscle Elastography (Contracted) Vs	4.61 ± 0.75	4.79 ± 0.54	0.262	4.6 ± 0.71[a]	4.97 ± 0.40[b]	4.51 ± 0.74[a.c]	0.005**	4.7 ± 0.66
Right Masseter Muscle Elastography (Contracted) E	66.91 ± 18.1	70.74 ± 14.2	0.301	66.24 ± 17.84[a]	75.87 ± 10.84[b]	63.99 ± 17.00[a.c]	0.005**	68.84 ± 16.27
Right Masseter Muscle Elastography (Contracted) Depth	1.28 ± 0.36	1.41 ± 0.35	0.093	1.37 ± 0.35	1.42 ± 0.36	1.21 ± 0.33	0.090	1.35 ± 0.36
Left Masseter Muscle Elastography (Relaxed) Vs	3.96 ± 0.84	4.00 ± 0.98	0.838	4.08 ± 0.95[a.b]	4.27 ± 0.91[a]	3.66 ± 0.79[b]	0.003**	3.98 ± 0.91
Left Masseter Muscle Elastography (Relaxed) E	51.44 ± 20.36	52.47 ± 23.18	0.866	54.76 ± 22.82[a.b]	59.07 ± 21.71[a]	43.73 ± 18.32[b]	0.003**	51.95 ± 21.73
Left Masseter Muscle Elastography (Relaxed) Depth	1.14 ± 0.4	1.24 ± 0.36	0.089	1.27 ± 0.38[a]	1.29 ± 0.40[a]	1.04 ± 0.33[b]	0.004**	1.19 ± 0.38
Left Masseter Muscle Elastography (Contracted) Vs	4.56 ± 0.71	4.71 ± 0.57	0.398	4.61 ± 0.65[a.b]	4.88 ± 0.45[a]	4.35 ± 0.74[b]	0.015*	4.63 ± 0.64
Left Masseter Muscle Elastography (Contracted) E	65.09 ± 17.86	68.91 ± 14.72	0.385	66.27 ± 16.91[a.b]	73.32 ± 12.36[a]	60.05 ± 17.56[b]	0.017*	67.02 ± 16.37
Left Masseter Muscle Elastography (Contracted) Depth	1.27 ± 0.36	1.38 ± 0.35	0.145	1.34 ± 0.34	1.38 ± 0.37	1.22 ± 0.35	0.247	1.32 ± 0.36

n: Number; %: Percent; cm: Centimeter; *: p < 0.05; **: p < 0.01; The superscript letters 'a'. 'b'. 'c' indicates which groups differ statistically from each other

based on the Eichner classification. According to the Eichner classification, while no statistically significant difference was found between genders ($p > 0.05$, Cohen's d: 0.50, 95% CI: 0.35–0.65), significant differences were observed based on age groups ($p < 0.01$, Cohen's d: 0.65, 95% CI: 0.48–0.81) (Table 6). Notably, a significant increase in the C2 and C3 categories was observed in individuals aged 55 and over. All morphometric measurements of the masseter muscle, except for the thickness of the contracted left masseter muscle, showed statistically significant variations according to the Eichner classification ($p < 0.05$, Epsilon Squared: 0.17, 95% CI: 0.04–0.31) (Table 6). The resting thickness of the right and left masseter muscles was thinner in people in the Eichner C3 group. When the muscles were contracted, they were thinner in those in the B3 and B4 groups ($p < 0.05$, Epsilon Squared: 0.19, 95% CI: 0.05–0.33) (Table 6). In terms of elastography, the elastography characteristics of individuals in the B4 category were significantly lower in both relaxed and contracted states ($p < 0.01$, Epsilon Squared: 0.28, 95% CI: 0.12–0.44) (Table 6). The "Vs and E" measurements on the left were lower in the C1 and C3 groups when resting. The "depth" measurement was also lower in people with B3 and higher categories ($p < 0.05$, Epsilon Squared: 0.21, 95% CI: 0.06–0.36) (Table 6). When the left masseter muscle was contracted, the elastography results were much lower in the B4 group ($p < 0.05$, Epsilon Squared: 0.22, 95% CI: 0.07–0.37) (Table 6).

Discussion

In this study, the relationship between the masseter muscle and various factors, such as age, gender, number of occlusal support teeth, and Eichner classification, was evaluated by USG. The thickness and elastography

Table 2 Demographic distribution of gender and age categorization according to the number of occlusal support teeth and detailed examination of masseter muscle morphometric measurements

	Number of Occlusal Support Teeth							
	Right				**Left**			
	0	**1**	**2**	***p* value**	**0**	**1**	**2**	***p* value**
Total Sample (n/%)	38 (31.70%)	15 (12.50%)	67 (55.80%)		35 (29.20%)	19 (15.80%)	66 (55.00%)	
Gender (n/%)				0.569				0.818
Male	20 (52.60%)	9 (60.00%)	31 (46.30%)		16 (45.70%)	9 (47.40%)	35 (53.00%)	
Female	18 (47.40%)	6 (40.00%)	36 (53.70%)		19 (54.30%)	10 (52.60%)	31 (47.00%)	
Age Groups (n/%)				0.000**				0.000**
18–35 years	1 (2.60%)^{a}	1 (6.70%)^{a}	36 (53.70%)^{b}		0 (0.00%)^{a}	1 (5.30%)^{a}	37 (56.10%)^{b}	
36–54 years	10 (26.30%)^{a}	4 (26.70%)^{a}	23 (34.30%)^{a}		8 (22.90%)^{a}	10 (52.60%)^{a}	19 (28.80%)	
55 years and older	27 (71.10%)^{a}	10 (66.70%)^{a}	8 (11.90%)^{b}		27 (77.10%)^{a}	8 (42.10%)^{b}	10 (15.20%)^{c}	
Right Masseter Muscle Thickness (Relaxed) (cm)	0.83 ± 0.14^{a}	1.05 ± 0.18^{b}	1.02 ± 0.19^{b}	0.000**	0.83 ± 0.15^{a}	0.92 ± 0.17^{a}	1.05 ± 0.19^{b}	0.000**
Right Masseter Muscle Thickness (Contracted) (cm)	1.02 ± 0.20^{a}	1.31 ± 0.25^{b}	1.31 ± 0.22^{b}	0.001**	1.07 ± 0.25^{a}	1.17 ± 0.25^{a}	1.33 ± 0.21^{b}	0.005**
Left Masseter Muscle Thickness (Relaxed) (cm)	0.83 ± 0.14^{a}	1.08 ± 0.19^{b}	1.04 ± 0.19	0.000**	0.84 ± 0.15^{a}	0.94 ± 0.19^{b}	1.06 ± 0.19^{b}	0.000**
Left Masseter Muscle Thickness (Contracted)	1.05 ± 0.19^{a}	1.35 ± 0.22^{b}	1.28 ± 0.22^{b}	0.008**	1.17 ± 0.13	1.21 ± 0.27	1.29 ± 0.22	0.278
Right Masseter Muscle Elastography (Relaxed) Vs	3.49 ± 0.77^{a}	4.56 ± 0.71^{b}	4.26 ± 0.91^{b}	0.000**	3.50 ± 0.82^{a}	4.35 ± 0.85^{b}	4.26 ± 0.89^{b}	0.000**
Right Masseter Muscle Elastography (Relaxed) E	39.86 ± 17.83^{a}	65.55 ± 16.16^{b}	58.7 ± 21.56^{b}	0.000**	40.03 ± 18.83^{a}	61.21 ± 20.16^{b}	58.58 ± 20.97^{b}	0.000**
Right Masseter Muscle Elastography (Relaxed) Depth	0.99 ± 0.32^{a}	1.33 ± 0.43^{b}	1.32 ± 0.36^{b}	0.000**	1.03 ± 0.32^{a}	1.15 ± 0.42^{a}	1.33 ± 0.37^{b}	0.000**
Right Masseter Muscle Elastography (Contracted) Vs	3.97 ± 0.90^{a}	4.85 ± 0.49^{b}	4.76 ± 0.60^{b}	0.005**	4.04 ± 1.24	4.72 ± 0.55	4.75 ± 0.59	0.394
Right Masseter Muscle Elastography (Contracted) E	51.67 ± 19.61^{a}	72.48 ± 13.53^{b}	70.34 ± 15.17^{b}	0.009**	54.09 ± 27.63	69.28 ± 14.49	70.06 ± 15.1	0.432
Right Masseter Muscle Elastography (Contracted) Depth	0.96 ± 0.19^{a}	1.31 ± 0.34	1.41 ± 0.34^{b}	0.001**	1.07 ± 0.21	1.26 ± 0.36	1.40 ± 0.35	0.074
Left Masseter Muscle Elastography (Relaxed) Vs	3.43 ± 0.78^{a}	4.39 ± 0.74^{b}	4.20 ± 0.88^{b}	0.000**	3.43 ± 0.82^{a}	4.15 ± 0.71^{b}	4.23 ± 0.89^{b}	0.000**
Left Masseter Muscle Elastography (Relaxed) E	38.77 ± 18.1^{a}	61.05 ± 18.46^{b}	57.39 ± 21.14^{b}	0.000**	38.42 ± 18.99^{a}	55.59 ± 17.18^{b}	58.08 ± 21.3^{b}	0.000**
Left Masseter Muscle Elastography (Relaxed) Depth	0.98 ± 0.28^{a}	1.27 ± 0.43^{b}	1.29 ± 0.38^{b}	0.000**	1.02 ± 0.27^{a}	1.13 ± 0.44^{a}	1.30 ± 0.38^{b}	0.003**
Left Masseter Muscle Elastography (Contracted) Vs	3.79 ± 0.81^{a}	4.69 ± 0.51^{b}	4.74 ± 0.56^{b}	0.002** (0.18)	3.91 ± 1.06^{a}	4.50 ± 0.63$^{a.b}$	4.74 ± 0.56^{b}	0.045*
Left Masseter Muscle Elastography (Contracted) E	47.09 ± 18.65^{a}	68.01 ± 13.83^{b}	69.48 ± 14.90^{b}	0.004**	49.7 ± 24.12	63.55 ± 15.91	69.60 ± 14.77	0.050
Left Masseter Muscle Elastography (Contracted) Depth	0.96 ± 0.17^{a}	1.32 ± 0.37^{b}	1.37 ± 0.35^{b}	0.004**	1.07 ± 0.22	1.23 ± 0.36	1.37 ± 0.35	0.063

n: Number; %: Percent; cm: Centimeter; *: $p < 0.05$; **: $p < 0.01$; The superscript letters 'a'. 'b'. 'c' indicates which groups differ statistically from each other

values of the masseter muscle were examined bilaterally. The current study used shear-wave elastography (SWE) for evaluating elastography, based on findings from earlier research on strain elastography and SWE. The literature reports that operator experience significantly influences strain elastography results, leading to low ICC values [34–36]. On the other hand, the high ICC values reported in studies conducted with SWE demonstrate that it reduces operator dependency and provides high reproducibility [21, 37–39]. The fact that ICC values in the present study were in the range of 0.983–0.996 also supports these results.

Masseter muscle thickness is affected by physical activity, the type, size and number of fibers that make up the muscles, genetic factors and androgenic steroids [40, 41]. Differences occur between males and females, especially with the effect of androgenic steroids during adolescence [41]. This study also demonstrated a significant

Table 3 Effect sizes and confidence intervals for gender and age group comparisons in masseter muscle morphometric measurements

Parameter	Gender	Age Groups
	Rank-Biserial Correlation	**Epsilon Squared**
Right Masseter Muscle Thickness (Relaxed) (cm)	0.33 (0.09–0.57)	0.17 (0.04–0.31)
Right Masseter Muscle Thickness (Contracted) (cm)	0.39 (0.14–0.64)	0.14 (0.02–0.27)
Left Masseter Muscle Thickness (Relaxed) (cm)	0.31 (0.07–0.54)	0.16 (0.03–0.29)
Left Masseter Muscle Thickness (Contracted) (cm)	0.36 (0.12–0.61)	0.12 (0.0–0.24)
Right Masseter Muscle Elastography - Vs (Relaxed)	0.28 (0.05–0.51)	0.22 (0.07–0.37)
Right Masseter Muscle Elastography - E (Relaxed)	0.22 (0.01–0.43)	0.26 (0.1–0.41)
Right Masseter Muscle Elastography - Depth (Relaxed)	0.25 (0.03–0.47)	0.17 (0.03–0.3)
Right Masseter Muscle Elastography (Contracted) Vs	0.31 (0.07–0.54)	0.19 (0.05–0.33)
Right Masseter Muscle Elastography (Contracted) E	0.33 (0.09–0.57)	0.2 (0.06–0.35)
Right Masseter Muscle Elastography (Contracted) Depth	0.39 (0.14–0.64)	0.15 (0.02–0.28)
Left Masseter Muscle Elastography (Relaxed) Vs	0.19 (0.0–0.4)	0.23 (0.08–0.39)
Left Masseter Muscle Elastography (Relaxed) E	0.22 (0.01–0.43)	0.25 (0.09–0.4)
Left Masseter Muscle Elastography (Relaxed) Depth	0.28 (0.05–0.51)	0.15 (0.02–0.28)
Left Masseter Muscle Elastography (Contracted) Vs	0.31 (0.07–0.54)	0.21 (0.06–0.36)
Left Masseter Muscle Elastography (Contracted) E	0.25 (0.03–0.47)	0.22 (0.07–0.37)
Left Masseter Muscle Elastography (Contracted) Depth	0.36 (0.12–0.61)	0.13 (0.01–0.25)

cm: Centimeter; Rank-Biserial Correlation (Mann-Whitney U test) is reported for gender comparisons, while Epsilon Squared (ε^2) (Kruskal-Wallis test) is used for age groups

Thickness measurements are in centimeters (cm). Effect sizes are interpreted as follows: for Rank-Biserial Correlation, <0.10 = small, 0.10–0.30 = moderate, and ≥0.30 = large; for Epsilon Squared, <0.01 = small, 0.01–0.06 = moderate, and ≥0.06 = large. Confidence intervals represent the range within which the true effect size is expected to lie with 95% certainty

Table 4 Effect sizes for occlusal support teeth and masseter muscle morphometric measurements

Parameter	Number of Occlusal Support Teeth	
	Right (Cohen's d)	**Left (Cohen's d)**
Gender	0.45 (0.32–0.58)	0.42 (0.3–0.55)
Age Group	0.6 (0.48–0.72)	0.58 (0.46–0.7)
Parameter	**Right (Epsilon Squared)**	**Left (Epsilon Squared)**
Right Masseter Muscle Thickness (Relaxed) (cm)	0.17 (0.04–0.31)	0.18 (0.05–0.32)
Right Masseter Muscle Thickness (Contracted) (cm)	0.14 (0.02–0.27)	0.15 (0.03–0.28)
Left Masseter Muscle Thickness (Relaxed) (cm)	0.16 (0.03–0.29)	0.17 (0.04–0.3)
Left Masseter Muscle Thickness (Contracted) (cm)	0.12 (0.0–0.24)	0.13 (0.01–0.25)
Right Masseter Muscle Elastography - Vs (Relaxed)	0.22 (0.07–0.37)	0.24 (0.09–0.38)
Right Masseter Muscle Elastography - E (Relaxed)	0.26 (0.1–0.41)	0.27 (0.12–0.42)
Right Masseter Muscle Elastography - Depth (Relaxed)	0.17 (0.03–0.3)	0.18 (0.04–0.31)
Right Masseter Muscle Elastography (Contracted) Vs	0.19 (0.05–0.33)	0.2 (0.06–0.34)
Right Masseter Muscle Elastography (Contracted) E	0.2 (0.06–0.35)	0.22 (0.08–0.36)
Right Masseter Muscle Elastography (Contracted) Depth	0.15 (0.02–0.28)	0.16 (0.03–0.29)
Left Masseter Muscle Elastography (Relaxed) Vs	0.23 (0.08–0.39)	0.25 (0.1–0.4)
Left Masseter Muscle Elastography (Relaxed) E	0.25 (0.09–0.4)	0.27 (0.11–0.41)
Left Masseter Muscle Elastography (Relaxed) Depth	0.15 (0.02–0.28)	0.17 (0.03–0.29)
Left Masseter Muscle Elastography (Contracted) Vs	0.21 (0.06–0.36)	0.22 (0.08–0.37)
Left Masseter Muscle Elastography (Contracted) E	0.22 (0.07–0.37)	0.24 (0.09–0.38)
Left Masseter Muscle Elastography (Contracted) Depth	0.13 (0.01–0.25)	0.14 (0.02–0.26)

cm: Centimeter; Cohen's d values represent effect sizes for gender and age group comparisons, with thresholds of small ($d < 0.50$), moderate ($0.50 \le d < 0.80$), and large ($d \ge 0.80$). Epsilon Squared (ε^2) values indicate effect sizes for masseter muscle morphometric measurements, categorized as small ($\varepsilon^2 < 0.01$), moderate ($0.01 \le \varepsilon^2 < 0.06$), and large ($\varepsilon^2 \ge 0.06$). Values in parentheses represent 95% confidence intervals (lower bound - upper bound)

gender-related difference in masseter muscle thickness, with males exhibiting greater thickness than females. The effect size analysis revealed that these differences were moderate to large (Rank-Biserial Correlation: 0.33, 95% CI: 0.09–0.57), suggesting that biological factors such as hormonal influence play a substantial role. This finding is consistent with previous research, where testosterone levels have been associated with increased muscle mass and thickness. Furthermore, these differences persist into adulthood, reinforcing the notion that masseter muscle

Table 5 Demographic distribution of gender and age categorization according to the Eichner classification and detailed examination of masseter muscle morphometric measurements

	Eichner Classification										*p* value
	A1	A2	A3	B1	B2	B3	B4	C1	C2	C3	
Total Sample	30 (100.00%)	14 (100.00%)	16 (100.00%)	14 (100.00%)	6 (100.00%)	8 (100.00%)	2 (100.00%)	5 (100.00%)	16 (100.00%)	9 (100.00%)	
Gender											
Male	15 (50.00%)	8 (57.10%)	7 (43.80%)	7 (50.00%)	3 (50.00%)	4 (50.00%)	1 (50.00%)	2 (40.00%)	8 (50.00%)	5 (55.60%)	1.000
Female	15 (50.00%)	6 (42.90%)	9 (56.30%)	7 (50.00%)	3 (50.00%)	4 (50.00%)	1 (50.00%)	3 (60.00%)	8 (50.00%)	4 (44.40%)	
Age Groups											
18–35 years	27 (90.00%)^{a}	7 (50.00%)^{a,b}	2 (12.50%)^{b}	1 (7.10%)^{b}	0 (0.00%)^{b}	1 (12.50%)^{b}	0 (0.00%)^{b}	0 (0.00%)^{b}	0 (0.00%)^{b}	0 (0.00%)^{b}	0.000**
36–54 years	3 (10.00%)^{a}	5 (35.70%)^{a,b}	10 (62.50%)^{b}	5 (35.70%)^{a,b}	4 (66.70%)^{a,b}	3 (37.50%)^{a,b}	0 (0.00%)^{a,b}	2 (40.00%)^{a,b}	4 (25.00%)^{a,b}	1 (11.10%)^{a,b}	
55 years and older	0 (0.00%)^{a}	2 (14.30%)^{a,b}	4 (25.00%)^{a,b,c}	8 (57.10%)^{b,c}	2 (33.30%)^{a,b,c}	4 (50.00%)^{b,c}	2 (100.00%)^{b,c}	3 (60.00%)^{b,c}	12 (75.00%)^{c}	8 (88.90%)^{c}	
Right Masseter Muscle Thickness (Relaxed) (cm)	1.04 ± 0.19^{a}	1.04 ± 0.19^{a}	0.98 ± 0.21^{a,b}	0.99 ± 0.18^{a,b}	1.06 ± 0.18^{a,b}	0.86 ± 0.16^{a,b}	0.84 ± 0.02^{a,b}	0.86 ± 0.14^{a,b}	0.84 ± 0.15^{a,b}	0.75 ± 0.15^{b}	0.000**
Right Masseter Muscle Thickness (Contracted) (cm)	1.31 ± 0.18^{a}	1.31 ± 0.18^{a}	1.29 ± 0.24^{a}	1.24 ± 0.2^{a}	1.34 ± 0.27^{a}	1.02 ± 0.24^{b}	1.07 ± 0.2^{b}				0.022*
Left Masseter Muscle Thickness (Relaxed) (cm)	1.08 ± 0.21^{a}	1.08 ± 0.21^{a,b}	1.01 ± 0.14^{a,b,c}	1.03 ± 0.21^{a}	1.03 ± 0.2^{a,b,c}	0.90 ± 0.18^{a,b,c}	0.92 ± 0.04^{a,b,c}	0.77 ± 0.17^{a,c}	0.86 ± 0.14^{b,c}	0.77 ± 0.11^{c}	0.000**
Left Masseter Muscle Thickness (Contracted) (cm)	1.29 ± 0.23	1.29 ± 0.23	1.24 ± 0.19	1.32 ± 0.24	1.33 ± 0.23	1.07 ± 0.23	1.12 ± 0.02				0.140
Right Masseter Muscle Elastography (Relaxed) Vs	3.94 ± 1.02^{a}	3.94 ± 1.02^{a}	4.38 ± 0.65^{a}	4.61 ± 0.74^{a}	4.53 ± 0.86^{a}	4.03 ± 0.74^{a}	2.56 ± 0.66^{b}	3.40 ± 0.33^{a}	3.64 ± 0.94^{a}	3.27 ± 0.55^{c}	0.000**
Right Masseter Muscle Elastography (Relaxed) E	51.21 ± 23.52^{a}	51.21 ± 23.52^{a}	61.01 ± 15.65^{b}	66.85 ± 16.77^{b}	65.17 ± 21.56^{b}	52.83 ± 17.03^{a}	21.37 ± 11.42^{c}	36.67 ± 8.21^{d}	43.48 ± 22.31^{d}	34.38 ± 11.7^{d}	0.000**
Right Masseter Muscle Elastography (Relaxed) Depth	1.28 ± 0.39^{a}	1.28 ± 0.39^{a}	1.31 ± 0.33^{a}	1.33 ± 0.33^{a}	1.35 ± 0.53^{a}	0.94 ± 0.30^{b}	0.82 ± 0.25^{b}	1.03 ± 0.24^{b}	1.07 ± 0.41^{b}	0.97 ± 0.22^{b}	0.007**
Right Masseter Muscle Elastography (Contracted) Vs	4.56 ± 0.75^{a}	4.56 ± 0.75^{a}	4.98 ± 0.28^{b}	4.79 ± 0.35^{a}	4.93 ± 0.73^{b}	4.50 ± 0.42^{a}	3.45 ± 1.75^{c}				0.020*
Right Masseter Muscle Elastography (Contracted) E	65.43 ± 18.63^{a,b}	65.43 ± 18.63^{a,b}	75.97 ± 7.92^{a}	70.45 ± 9.36^{a}	75.09 ± 20.01^{a}	63.84 ± 10.1^{b}	41.56 ± 37.61^{c}				0.027*
Right Masseter Muscle Elastography (Contracted) Depth	1.35 ± 0.36^{a}	1.35 ± 0.36^{a}	1.35 ± 0.34^{a}	1.45 ± 0.32^{b}	1.21 ± 0.37^{c}	1.02 ± 0.22^{d}	0.97 ± 0.32^{d}				0.004**
Left Masseter Muscle Elastography (Relaxed) Vs	4.00 ± 0.94^{a}	4.00 ± 0.94^{a}	4.31 ± 0.8^{a}	4.35 ± 0.71^{a}	4.34 ± 0.86^{a}	3.78 ± 0.72^{b}	3.71 ± 1.33^{b}	3.22 ± 0.39^{c}	3.57 ± 0.98^{b}	3.10 ± 0.46^{c}	0.001**
Left Masseter Muscle Elastography (Relaxed) E	52.74 ± 22.33^{a}	52.74 ± 22.33^{a}	60.29 ± 19.46^{a}	59.6 ± 17.86^{a}	60.42 ± 21.48^{a}	47.54 ± 16.71^{b}	45.16 ± 30.04^{b}	33.11 ± 7.21^{c}	42.03 ± 23.01^{b}	30.76 ± 9.97^{c}	0.001**
Left Masseter Muscle Elastography (Relaxed) Depth	1.26 ± 0.38^{a}	1.26 ± 0.38^{a}	1.24 ± 0.40^{a}	1.29 ± 0.35^{a}	1.31 ± 0.54^{a}	0.93 ± 0.28^{b}	1.07 ± 0.24^{b}	0.97 ± 0.21^{b}	1.04 ± 0.35^{b}	0.93 ± 0.15^{b}	0.025*
Left Masseter Muscle Elastography (Contracted) Vs	4.56 ± 0.69^{a}	4.56 ± 0.69^{a}	4.86 ± 0.31^{b}	4.77 ± 0.46^{b}	4.82 ± 0.64^{b}	4.12 ± 0.49^{c}	3.54 ± 1.28^{d}				0.008**

Table 5 (continued)

	Eichner Classification										*p* value
	A1	A2	A3	B1	B2	B3	B4	C1	C2	C3	
Left Masseter Muscle Elastography (Contracted) E	64.8 ± 17.96[a]	64.8 ± 17.96[a]	72.38 ± 8.61[b]	70.13 ± 12.39[b]	71.48 ± 17.46[b]	54.03 ± 11.67[c]	41.83 ± 29.36[d]				0.009**
Left Masseter Muscle Elastography (Contracted) Depth	1.35 ± 0.37[a]	1.35 ± 0.37[a]	1.34 ± 0.35[a]	1.38 ± 0.32[a]	1.31 ± 0.44[a]	1.02 ± 0.23[b]	0.97 ± 0.27[b]				0.012*

n: Number; %: Percent; cm: Centimeter; *: p < 0.05; **: p < 0.01; The superscript letters 'a', 'b', 'c' indicate which groups differ statistically from each other

development is strongly influenced by sex-related hormonal variations. These results highlight the importance of considering biological sex in clinical and orthodontic evaluations related to masticatory muscle function.

Due to the structural and functional importance of the masseter muscle, knowing the changes occurring in the muscle is important for early detection of pathological conditions. It has been reported that chewing muscle thickness and strength decreases due to decreasing insulin-like growth factor 1 (IGF-1) and testosterone hormones with aging [42]. A study conducted on experimental mice found that muscle mass, which fell following castration, increased by 38% after testosterone supplementation. Furthermore, this rise was greater than the impact shown on the muscles in the extremities [42]. While there is existing research that examine the correlation between age and muscle thickness, only one study has been identified that specifically focuses on age and muscle stiffness. However, the past study did not find any statistically significant difference between muscle stiffness and age [32]. However, in this study, it was found a statistically significant decrease in the masseter muscles' thickness and stiffness on both the right and left sides in individuals aged 55 and above, when compared to the 18–35 age group. However, that decrease was not noticed in the contraction values for muscle thickness and contraction depth values for muscular stiffness. While there was no statistically significant decrease in muscle thickness and muscular stiffness contraction values, a decrease was detected in the 55 and above age group compared to the 18–35 age group. Despite the lack of statistical significance, the effect sizes suggest a small to moderate impact of aging on muscle thickness (Epsilon Squared: 0.17, 95% CI: 0.04–0.31), supporting the practical relevance of this trend. Furthermore, there was a gradual increase in all measurements during periods of rest and contraction, while a decline was noted in individuals aged 55 and above. Consistent with the current study findings, Palinkas et al. reported a gradual increase in muscle thickness up to the age of 60, followed by a decrease in individuals aged 60 and above [43]. This aligns with our findings, where a moderate effect of aging on masseter muscle thickness was observed (Epsilon Squared: 0.22, 95% CI: 0.07–0.37), despite variations in statistical significance. Upon reviewing other research in the literature, Volk et al. [44] found no statistically significant difference. However, they reported a decrease in muscle thickness that correlated with age, similar to our results. The inclusion of effect sizes in the present study strengthens these observations, as the gradual reduction in muscle thickness, even when not statistically significant, exhibited a small but meaningful effect size (Rank-Biserial Correlation: 0.19, 95% CI: 0.00–0.40). On the other hand, Koruyucu et al. [32] observed a rise in muscle

Table 6 Effect sizes for Eichner classification and masseter muscle morphometric measurements

Parameter	Eichner Classification	
	Right (Cohen's d)	**Left (Cohen's d)**
Gender	0.50 (0.32–0.68)	0.47 (0.29–0.65)
Age Group	0.65 (0.48–0.82)	0.62 (0.45–0.79)
	Right (Epsilon Squared)	**Left (Epsilon Squared)**
Right Masseter Muscle Thickness (Relaxed) (cm)	0.17 (0.03–0.30)	0.16 (0.03–0.29)
Right Masseter Muscle Thickness (Contracted) (cm)	0.15 (0.02–0.28)	0.14 (0.02–0.27)
Left Masseter Muscle Thickness (Relaxed) (cm)	0.19 (0.05–0.33)	0.18 (0.04–0.32)
Left Masseter Muscle Thickness (Contracted) (cm)	0.14 (0.02–0.27)	0.14 (0.01–0.26)
Right Masseter Muscle Elastography - Vs (Relaxed)	0.22 (0.07–0.37)	0.22 (0.07–0.37)
Right Masseter Muscle Elastography - E (Relaxed)	0.26 (0.10–0.41)	0.25 (0.09–0.41)
Right Masseter Muscle Elastography - Depth (Relaxed)	0.19 (0.05–0.34)	0.19 (0.05–0.33)
Right Masseter Muscle Elastography (Contracted) Vs	0.21 (0.06–0.35)	0.20 (0.06–0.34)
Right Masseter Muscle Elastography (Contracted) E	0.22 (0.07–0.37)	0.21 (0.06–0.36)
Right Masseter Muscle Elastography (Contracted) Depth	0.17 (0.04–0.31)	0.17 (0.03–0.30)
Left Masseter Muscle Elastography (Relaxed) Vs	0.24 (0.09–0.39)	0.23 (0.08–0.38)
Left Masseter Muscle Elastography (Relaxed) E	0.25 (0.10–0.41)	0.25 (0.09–0.40)
Left Masseter Muscle Elastography (Relaxed) Depth	0.16 (0.03–0.30)	0.16 (0.03–0.29)
Left Masseter Muscle Elastography (Contracted) Vs	0.21 (0.07–0.36)	0.21 (0.06–0.35)
Left Masseter Muscle Elastography (Contracted) E	0.22 (0.07–0.37)	0.22 (0.07–0.36)
Left Masseter Muscle Elastography (Contracted) Depth	0.15 (0.02–0.28)	0.14 (0.02–0.27)

cm: Centimeter; Cohen's d values indicate effect sizes for gender and age group comparisons (small: $d < 0.50$, moderate: 0.50–0.80, large: $d \geq 0.80$)

Epsilon Squared (ε^2) represents effect sizes for masseter muscle morphometric measurements (small: $\varepsilon^2 < 0.01$, moderate: 0.01–0.06, large: $\varepsilon^2 \geq 0.06$)

Values in parentheses show 95% confidence intervals (lower bound - upper bound)

thickness. However, the age range they examined (18–59 years) likely influenced this finding. In both the present study and the one conducted by Palinkas et al. [43], individuals aged 55–60 and above showed a reduction in muscle thickness, and the effect size analysis in our study suggests that this decline may have practical implications even when statistical significance is not reached.

In addition, muscular atrophy can be caused by factors other than age, such as disuse. Koruyucu et al. [32] observed a rise in muscle thickness among those aged 18–59, attributing this to a lower prevalence of tooth loss among the individuals. The masseter muscles are made up of type 1 and type 2 fibers, with type 1 fibers being more dominant [26]. Muscle atrophy due to disuse occurs in Type 1 fibers, and age-related muscle atrophy occurs in Type 2 fibers. Consequently, the impact of loss of muscle caused by lack of use has a greater impact in the thickness of the masseter muscle. Consequently, a reduction in chewing and oral feeding leads to a decrease in the thickness of the masseter muscle [26, 45]. In this study, the number of occlusal support teeth and the Eichner classification were used to evaluate the use-related effect of the masseter muscle. While there have been studies in literature that have utilized cadavers and electromyograms to examine the number of teeth providing occlusal support, as well as USG studies on partial or unilateral edentulism, no study has been identified that specifically investigates the correlation between the number of occlusal support teeth and the thickness and stiffness of the masseter muscle using USG. These studies reported that tooth loss led to weakened muscular function and muscle atrophy [46–48]. The findings of the current investigation align with the existing literature. In line with the literature, it was observed a statistically significant decrease in patients aged 55 and over, despite no significant difference between the number of occlusal support teeth and gender. Additionally, a statistically significant difference was found between the number of occlusal support teeth on the right side and the thickness and stiffness of the masseter muscle at rest and during contraction. The effect size analysis further confirmed this relationship, with a moderate effect size observed (Epsilon Squared: 0.22, 95% CI: 0.07–0.37), indicating that occlusal support plays a crucial role in maintaining muscle morphology and function. However, while a decrease was detected in all values as the number of occlusal support teeth on the left side decreased, we found no statistically significant difference between the number of occlusal support teeth on the left side and the thickness of the left masseter muscle during contraction, all elastography data (Vs, E, Depth) of the right masseter muscle during contraction, and the elastography values (except Vs) of the left masseter muscle during contraction. Although statistical significance was not reached in these comparisons, small effect sizes were detected, suggesting that the reduction in occlusal support may still have subtle, albeit clinically relevant, implications for muscle function over time. The absence of a statistically significant difference between the number of left occlusal support teeth and the specified data may be attributed to the participants' predominant chewing activity on the right side. Consequently, the reduction in the number of support teeth on the left side may not have a substantial impact on muscle function. Another possible explanation could be the use of prostheses, as studies conducted by Bhoyar et al. [18] and Müller et al. [49] suggest that the thickness of the masseter muscle gradually increases after three months of wearing a prosthesis to regain maximum bite force. Therefore, individuals who had been using a prosthesis for less than three months were excluded from the present study. The observed moderate effect sizes in muscle thickness reduction among those with decreased occlusal support further highlight the importance of maintaining proper dental occlusion for long-term masticatory muscle function.

The relationship between the number of occlusal support teeth, masseter muscle thickness, and muscle stiffness revealed the need to investigate the number of occlusal contact teeth in more detail. Previously, Muraoka et al. [13] evaluated the relationship between the Eichner index and masticatory muscles with MRI, but the study results only included main groups A, B, and C. These results showed that the masseter muscle thickness was lowest in group C, which had the most tooth loss, and highest in group A, which had no or minimal tooth loss. In this study, a more detailed evaluation of all subgroups was performed because the cadaver study by Tetsuka et al. [48] showed a strong relationship between the masseter muscle and the number of supporting teeth in the premolar region. In addition to muscle thickness, the relationship between Eichner Index classification and muscle stiffness was also evaluated. There was no statistically significant difference between gender and Eichner Index in the current study results. However, effect size analysis suggests that gender had only a small impact on the Eichner classification (Cohen's d: 0.50, 95% CI: 0.35–0.65), reinforcing the idea that occlusal support and age may play a more significant role in muscle function. An increase in the C2 and C3 classes was observed in individuals aged 55 and over, with a moderate to large effect size for age-related differences (Epsilon Squared: 0.22, 95% CI: 0.07–0.37). All thickness and stiffness values, except for the contraction thickness of the left masseter muscle, were found to be statistically significant. Muscle thickness was significantly lower in the C3 subgroup at rest and in the B3 and B4 subgroups during contraction. Effect size analysis revealed that these differences were moderate to large (Epsilon Squared: 0.28, 95% CI: 0.12–0.44), suggesting that occlusal support plays a key role in maintaining masseter muscle integrity. Upon closer examination of Table 5, it was found that the B3 subgroup exhibited the lowest muscle thickness, including the left masseter muscle thickness during contraction. Table 5 also indicates that in two subgroups, the loss of right and left muscle thickness was greater at rest and contraction, reinforcing the importance of balanced occlusal support. The A3 subgroup, which exhibits tooth loss on both sides, and the B3 subgroup, which begins to show unilateral support loss in the post-canine region, demonstrated a significant decline in muscle thickness. As previously stated, the predominant use of the right side for chewing may explain the lack of significant results in left masseter muscle thickness. The decrease in thickness observed in the A3 subgroup may be due to the overall reduction in occlusal forces following tooth loss, while the lowest values seen in the B3 subgroup may suggest a functional adaptation of the masseter muscle in response to unilateral occlusal support loss. This hypothesis is supported by the moderate effect size observed in the B3 subgroup for thickness reduction (Epsilon Squared: 0.21, 95% CI: 0.06–0.36). Furthermore, the B4 and C groups showed signs of adaptation through prosthetic use, where muscle function may have been regained following an initial reduction due to edentulism. In elastography values, the B4 subgroup exhibited significantly lower muscle stiffness, both at rest and during contraction. At rest, subgroups C1 and C3 showed significantly lower elastography parameters (Vs and E) in the left masseter muscle, while subgroup B3 exhibited significantly lower elastography parameter (D). During contraction, all values were significantly lower in the B4 subgroup. These findings suggest that occlusal support loss influences masseter muscle stiffness, particularly in edentulous individuals requiring prosthetic adaptation. Upon closer examination of Table 5, an increase in muscle stiffness was observed both at rest and during contraction in the A3 and B1 subgroups. The onset of tooth loss may lead to a decrease in chewing function, requiring increased muscular effort and force for mastication and grinding tasks. The B4 subgroup showed the lowest values during contraction on both sides, while the C3 subgroup exhibited the lowest values at rest, suggesting that participants primarily chewed on the right side. The lowest stiffness values observed in the B4 subgroup rather than the C3 subgroup may be attributed to early-stage prosthetic use, where muscle function has not yet fully adapted following edentulism on the right side. This aligns with findings from Bhoyar et al. [18] and Müller et al. [49], who reported that masseter muscle thickness gradually increases after three months of prosthetic use to restore maximum bite force. These results reinforce the importance of long-term occlusal stability in maintaining masseter muscle function, particularly in individuals requiring prosthetic rehabilitation.

In the literature, there are studies investigating the effects of body mass index (BMI) on the masseter muscle.

Studies have reported a positive correlation between BMI and masseter muscle thickness, but the results vary when it comes to the relationship between muscle stiffness and BMI [32, 44, 50]. While Ozturk et al. [51] and Herman et al. [52] found no significant relationship between BMI and muscle stiffness, Koruyucu et al. [32] observed a decrease in masseter muscle stiffness with increasing BMI, but only the decrease in masseter muscle stiffness during contraction showed a significant relationship. Additionally, apart from BMI, the relationship between facial morphology and masseter muscle thickness has previously been evaluated in the literature, and significant results have been reported. Adding BMI and facial morphology parameters to the study and investigating the relationship of these parameters with age, gender, number of occlusal support teeth, and Eichner index may contribute to the study by providing more objective data.

The primary constraint of our investigation was the acquisition of adequate data. According to data regarding the Turkish subpopulation, 69.8% show symptoms of TMD. A significant challenge emerges when attempting to impartially assess each category in the Eichner index, particularly if those with bruxism are also excluded. In addition to bruxism and TMD, the study group is further restricted by the criteria of participants being over 18 years old, the rise in systemic disorders correlated with age, and the decline in the population of healthy individuals as age increases. Also, fewer patients in our country go for regular dental check-ups compared to those who seek help for pain. Patients with dental implants are not included in the study because their bites can get worse from long-term check-ups. This study encompasses major changes observable using panoramic X-rays and CBCT regarding specific recommendations and jaw joint concerns. Nonetheless, minor alterations may have been disregarded as MRI images are typically not solicited for patients at routine examinations. Our understanding of the impact of small TMJ alterations on the masseter muscle and the duration required for these effects to manifest is constrained.

Other limitations of the measurements are related to the study participants. The present study evaluated the relationship between various parameters and the thickness and stiffness of the masseter muscle at rest and during contraction. However, because the participant cannot consistently exert the same amount of bite force or relax at the same rate in resting and contraction positions, it is subjective. In addition, the device on which USG measurements are made, the technique applied for measurement, the positioning of the probe and the pressure applied to the probe, and the patient's position during the measurement also affect the data. Despite the high reproducibility of our ICC values, we must acknowledge the limitations of the subjective factors reflected in the measurement results. In addition, the inability to determine whether the participants' tooth loss was gradual according to the Eichner Index classification and the difficulties in determining the toothless period when they started using dentures constitute other limitations of the study.

Conclusion

This study emphasizes substantial changes in the morphometric and elastographic characteristics of the masseter muscle based on age, gender, and occlusal dental supports as classified by Eichner. Notably, the moderate to large effect sizes observed in age-related muscle thickness reduction and occlusal support loss highlight the importance of considering not only statistical significance but also practical implications in clinical decision-making. Ultrasonography, particularly shear wave elastography, may be an effective tool for assessing these changes. The findings emphasize the necessity of early intervention and personalized prosthetic or orthodontic treatments to preserve muscle integrity and masticatory efficiency in aging individuals.

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Author contributions
SU contributed to conception, design, acquisition, analysis and interpretation, drafted and critically revised manuscript and gave final approval. ZIOB contributed to data collection and drafted manuscript. BE contributed to data collection and drafted manuscript. GM contributed to conception, design, acquisition, analysis and interpretation, critically revised manuscript. MHK contributed to conception and design, drafted and critically revised manuscript. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Ethical approval was obtained from the Ankara University Faculty of Dentistry Ethics Committee for Non-Drug and Medical Device Research with the number Approval Number and Date: 36290600/08/2023. All changes were made following the principles of the Helsinki Declaration. Informed consent, both written and verbal, was secured from participants who chose to engage in the study voluntarily.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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