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RESEARCH ARTICLE

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Ultrasonographic assessment of perceived stress, temporomandibular disorders, and sociodemographic influences on masseter, temporalis, and digastric muscle thickness

Berkant Sezer DDS, MSc[a], Gamze Coşan Ata DDS, MSc[b], Aysu Şahin DDS[c], Doğa Doğan DDS[c] and Toprak Efe Ersöz DDS[d]

[a]Department of Pediatric Dentistry, School of Dentistry, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye; [b]Department of Dentomaxillofacial Radiology, School of Dentistry, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye; [c]Private Practice, Bursa, Türkiye; [d]Private Practice, Çanakkale, Türkiye

ABSTRACT

Objective: This study examined the associations between perceived stress, sociodemographic and health-related factors, temporomandibular disorders (TMD), and masticatory muscle thickness assessed by ultrasonography.

Methods: Forty participants were classified into high- and normal-stress groups. Perceived stress, TMD status, and ultrasonographic thickness of the masseter, anterior temporalis, and anterior digastric muscles were assessed.

Results: Higher perceived stress was associated with greater TMD prevalence and reduced anterior digastric muscle thickness. Male participants showed greater masseter and digastric muscle thickness. In regression analyses, female gender and high perceived stress were associated with reduced anterior digastric thickness. However, group differences in TMD prevalence and the absence of bruxism assessment limit causal inference.

Conclusion: Perceived stress and gender were associated with anterior digastric muscle thickness in this exploratory, cross-sectional sample of young adults.

KEYWORDS
Ultrasonography; USG; imaging; stress; temporomandibular joint

Introduction

Perceived stress reflects an individual's subjective evaluation of life situations as demanding or overwhelming [1]. It represents an adaptive response to challenges and can manifest through psychological, behavioral, and physiological alterations [2]. Unlike stressors themselves, perceived stress focuses on how stressful situations are interpreted rather than on specific events [1,2]. High perceived stress levels have been associated with conditions such as depression, anxiety, and various somatic symptoms [3,4]. University students are particularly susceptible due to academic pressure, social expectations, and financial concerns [5].

The masticatory muscles – comprising the masseter, temporalis, medial pterygoid, and lateral pterygoid – are essential for mandibular movement and oral function, and their morphology can be influenced by age, sex, genetic predisposition, functional demand, and parafunctional behaviors such as awake or sleep bruxism [6–11]. Systemic conditions, nutritional status, psychological and psychiatric factors, and temporomandibular disorders (TMDs) may alter muscle thickness through changes in neuromuscular activity and muscle tone [12–18]. Importantly, psychosocial stress and TMDs frequently coexist, and higher stress levels have been consistently associated with increased TMD symptom severity [14,15]. Because these conditions frequently overlap, observed associations between perceived stress and muscle morphology may partly reflect TMD-related neuromuscular alterations rather than stress alone. Moreover, awake and sleep bruxism – currently conceptualized as behavioral phenomena – constitute major determinants of masseter and temporalis hypertrophy and are widely recognized as key confounding factors in research examining stress-related or TMD-related changes in masticatory muscle morphology [10, 11].

The anterior digastric muscle is of particular interest due to its role in mandibular depression and hyoid bone elevation, its dual-bellied structure, and its reliance on fine neuromuscular coordination rather than force generation [19–21]. Its activity may be influenced by occlusal dynamics, temporomandibular joint (TMJ) status, and autonomic regulation – factors that themselves can be modulated under psychological stress [19–23].

CONTACT Berkant Sezer DDS, MSc dt.berkantsezer@gmail.com; berkant.sezer@comu.edu.tr Department of Pediatric Dentistry, School of Dentistry, Çanakkale Onsekiz Mart University, Cumhuriyet Mah. Sahilyolu Cd., No:5, Çanakkale 17100, Türkiye

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Although direct mechanistic pathways linking perceived stress to digastric morphology have not been established, some authors suggest that low-load stabilizing muscles may be more sensitive to fluctuations in autonomic tone and central motor regulation than high-load elevator muscles [21, 22]. However, this proposed mechanism remains speculative and cannot be confirmed without longitudinal data or concurrent functional assessments such as electromyography (EMG). Therefore, any potential association between perceived stress and anterior digastric thickness should be interpreted cautiously as a hypothesized and indirect relationship rather than a demonstrated physiological consequence. Current knowledge remains largely correlational and requires longitudinal and mechanistic research to clarify these proposed pathways.

Several imaging modalities, including EMG, computed tomography (CT), magnetic resonance imaging (MRI), and ultrasonography, are widely employed to evaluate the morphology and function of masticatory muscles and TMJ. These techniques provide valuable data on muscle thickness, cross-sectional area, and dynamic activity [24–29]. Among these, ultrasonography is particularly advantageous because it is non-invasive, radiation-free, and suitable for repeated assessments. It provides reliable measurements of superficial muscles, making it well suited for evaluating the masseter and, to a lesser extent, the anterior temporalis and anterior belly of the digastric [27, 30–33].

Based on these considerations, the present study aimed to investigate the associations between perceived stress – along with sociodemographic and health-related factors – and the ultrasonographic thickness of the masseter, anterior temporalis, and anterior digastric muscles. The study population consisted of two groups of individuals working under similar conditions: one with normal perceived stress levels and another with high levels of perceived stress. In this context, the primary objective was to examine whether perceived stress levels are associated with variations in masticatory muscle thickness, whereas sociodemographic characteristics, health-related variables, and TMD status were evaluated as secondary exploratory factors. The null hypothesis stated that perceived stress is not significantly associated with the thickness of the evaluated masticatory muscles.

Subjects and methods

Ethical approval for this cross-sectional observational study was obtained from the Non-Interventional Clinical Research Ethics Committee of Çanakkale Onsekiz Mart University on May 8 2024 (protocol no. 02/2024–14). All procedures adhered to the ethical principles outlined in the Declaration of Helsinki. The study was carried out between May and September 2024 at the School of Dentistry, Çanakkale Onsekiz Mart University. Prior to participation, all potential participants received detailed oral and written information regarding the study, and written informed consent was obtained. The consent form included comprehensive explanations to ensure participants fully understood the nature and scope of the research. Participation was entirely voluntary, with the right to withdraw at any stage without consequence.

Sample size calculation

The required sample size was calculated using G*Power software (version 3.1.9.2; Heinrich Heine University, Düsseldorf, Germany). Data from the study by Jokubauskas et al. [34], which investigated the relationship between possible bruxism and perceived stress among undergraduate students, were used as reference values. Based on their reported means and standard deviations (21 ± 6.5 and 17 ± 6.7), and applying a significance level of $\alpha = 0.05$ with a statistical power of $1 - \beta = 0.98$, the minimum required sample size was determined to be 33 participants. To account for an anticipated 20% dropout rate, the final target sample size was set at a minimum of 40 participants, with 20 individuals allocated to each group (normal and high perceived stress levels).

Primary and secondary outcomes

The primary outcome of this study was the comparison of ultrasonographically measured thicknesses of the masseter, anterior temporalis, and anterior digastric muscles between individuals with normal and high perceived stress levels. Secondary exploratory outcomes included evaluating whether sociodemographic variables, health-related characteristics, and the presence or absence of TMDs influenced masticatory muscle thickness.

Study population

Perceived stress levels and their associated psychological and physiological changes may be comparable among individuals working under similar conditions within the same environment [35]. To minimize the influence of external confounding variables, the present study was restricted to participants with homogeneous workplace settings. Accordingly, the study population comprised senior clinical dental students enrolled at the School of Dentistry, Çanakkale Onsekiz Mart University, during

the 2023–2024 academic year. Inclusion criteria required participants to fully understand the study procedures and voluntarily provide written informed consent. Participants were also required to present with complete dentition in all four quadrants and to be free of severe malocclusion. Exclusion criteria consisted of: (1) the presence of musculoskeletal disorders affecting the head and neck region; (2) a diagnosis of or treatment for neoplastic disease in this region within the previous six months; (3) a history of surgical procedures involving the head and neck; (4) severe malocclusion (e.g., Angle Class II or III, open bite, or crossbite); and (5) ongoing active orthodontic treatment.

First phase of the study

In the first phase of the study, the principal investigator (B.S.) provided potential participants with detailed information about the research in a classroom setting. After obtaining informed consent, participants completed a two-part questionnaire. The first section gathered sociodemographic and health-related information, including age, gender, parental education level and income, living arrangements during undergraduate education (e.g., dormitory or home), presence of diagnosed chronic diseases (excluding musculoskeletal disorders affecting the head and neck), regular medication use (excluding drugs related to musculoskeletal conditions), and regular tobacco or alcohol consumption.

The second section assessed perceived stress using the 14-item Perceived Stress Scale (PSS-14), which evaluates the extent to which individuals perceive their lives as unpredictable, uncontrollable, and overloaded during the past month [36]. This Likert-type instrument consists of 14 items scored from 0 (never) to 4 (very often), with items 4, 5, 6, 7, 9, 10, and 13 reverse scored. The total score ranges from 0 to 56, with higher scores reflecting greater perceived stress. Scores between 0 and 35 are interpreted as indicative of normal stress levels, suggesting effective stress management and coping strategies, whereas scores between 36 and 56 represent high perceived stress, reflecting insufficient stress control and maladaptive coping [37, 38]. The Turkish version of the PSS-14 has been validated among university students by Kaya et al. [39], and its reliability and validity – both in the long and short forms – were further confirmed by Eskin et al. [40]. In the present study, participants were dichotomized into normal and high stress groups based on these validated thresholds, rather than the traditional three-category model (low/moderate/high), because low-stress individuals were minimally represented in this dental student cohort. Accordingly, low and moderate classifications were combined into the normal stress group, ensuring balanced group sizes for meaningful statistical comparisons.

Data were entered into Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). Of the 77 students initially invited, 8 declined participation and 10 did not meet the inclusion criteria. The PSS-14 scores of the remaining 59 students were analyzed by an independent, blinded statistician who had no access to sociodemographic or health-related data. Based on these results, participants were categorized into two groups: high and normal perceived stress levels. The statistician then assigned anonymous codes to 31 students in the high-stress group and 28 students in the normal-stress group. The assigned codes were entered into a randomization software program (www.randomizer.org), and 20 participants were randomly selected from each group. The randomization results were communicated to the principal investigator, who subsequently accessed the identity information of the selected participants and invited them to participate in the second phase of the study. At this stage, two participants from each group declined to continue, and the same randomization procedure was repeated to replace them. Ultimately, each group comprised 20 participants, yielding a final study sample of 40 individuals.

Second phase of the study

Participants selected for the second phase of the study were invited to the clinic for extraoral and intraoral examinations, as well as ultrasonographic imaging. Before these procedures, participants were re-informed about the study and asked to complete the Fonseca Anamnestic Index (FAI), a 10-item self-reported questionnaire widely used for TMD screening [41]. The FAI evaluates symptoms such as TMJ pain, headaches, back pain, pain during chewing, parafunctional habits, joint sounds, occlusal abnormalities, and emotional stress. Responses are scored as “Yes” (10 points), “Sometimes” (5 points), or “No” (0 points), with total scores ranging from 0 to 100:

- 0–15 indicates absence of TMD,
- 20–40 indicates mild dysfunction,
- 45–65 indicates moderate dysfunction, and
- 70–100 indicates severe dysfunction [42].

Following questionnaire completion, standardized TMJ examinations were performed by a single experienced maxillofacial surgeon using the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) [43] and its Turkish version, which has demonstrated strong

reliability and diagnostic accuracy in Turkish populations [44]. The clinical protocol included patient history, palpation of the TMJs and masticatory muscles, auscultation of joint sounds, and assessment of mandibular range of motion. To ensure consistency, the examiner was calibrated by performing TMJ evaluations on 10 individuals not included in the study, with a two-week interval, yielding an intra-examiner kappa coefficient of 0.97.

Following the clinical examination, ultrasonographic imaging of the masseter, anterior temporalis, and anterior digastric muscles was performed. Examinations were conducted using an ARIETTA 65 ultrasound system (Hitachi, Tokyo, Japan) equipped with an L64 linear transducer operating at a pulse frequency of 5–18 MHz. A standardized B-mode grayscale setting was applied for musculoskeletal assessments. All measurements were obtained with participants in a relaxed supine position (Figure 1). Initially, the facial area and body were relaxed, and participants were instructed to gently approximate the upper and lower lips. Muscle thickness was then measured in both resting and clenching positions. All ultrasonographic evaluations were performed by a single experienced dentomaxillofacial radiologist (G.C.A.) who was blinded to the clinical TMJ examination results. To ensure examiner calibration, ultrasonographic assessments were repeated in 10 participants not included in the study, with a two-week interval between sessions. The intra-examiner kappa value was calculated as 0.94, confirming excellent reliability.

For the masseter muscle, the probe was positioned over the most prominent part of the muscle, aligned parallel to the long axis of the zygomatic arch at the level of the mandibular ramus (Figure 1(A)). Thickness measurements were obtained in both resting and clenching positions, and linear values were recorded from the anterior, middle, and posterior regions. For the anterior temporalis muscle, the probe was placed transversely between the superior and lateral border of the orbit and the anterior edge of the hairline, with measurements taken from the thickest portion of the anterior horizontal band (Figure 1(B)). For the anterior digastric muscle, the probe was positioned transversely on the outer surface of the submandibular region, and bilateral thickness measurements were recorded (Figure 1(C)). Muscle thickness was defined as the maximum distance between the outer and inner fasciae.

All measurements were obtained bilaterally, and the asymmetry index developed by Alkaya et al. [31] was applied to evaluate differences between the left and right sides: [(Right muscle thickness – Left muscle thickness)/(Right muscle thickness + Left muscle thickness)] x 100.

To minimize measurement error, each assessment was repeated three times at five-minute intervals, and the mean value of the three measurements was used for statistical analysis. Rest periods were provided between measurements to prevent muscle fatigue. For the masseter muscle, three measurements were taken from the anterior, middle, and posterior regions in both resting and clenching positions, and the mean value was recorded as the final thickness. For the anterior temporalis and anterior digastric muscles, the maximum thickness was measured and recorded.

Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics, version 26 (IBM Corp., Armonk, NY, USA). The significance level was set at $p < .05$ for all tests. The Shapiro – Wilk test was applied to assess the normality of continuous variables. Numerical perceived stress scores, clenching and resting thicknesses of the right masseter, clenching and resting thicknesses of the masseter and anterior temporalis muscles, and age were normally distributed ($p > .05$). In contrast, the thicknesses of the right and left anterior digastric muscles ($p < .001$ and $p = .001$, respectively), clenching and resting thicknesses of the right anterior temporalis ($p = .012$ and $p = .002$, respectively), and categorical variables



Figure 1. Clinical demonstration of standardized probe positioning for ultrasonographic assessment of the masseter (A), anterior temporalis (B), and anterior digastric (C) muscles.

such as parental education level, income, residence, smoking and alcohol use, regular medication use, systemic disease, FAI score categories, and perceived stress groups were not normally distributed ($p < .001$ for all).

Quantitative data were summarized as mean ± standard deviation (SD), frequencies (%), or minimum – maximum values, as appropriate. Comparisons of categorical variables between the high and normal perceived stress groups were conducted using Pearson's Chi-square test or Fisher's exact test when required. For continuous variables, the choice of statistical test was guided by distributional characteristics and study design. Independent samples t-tests were used for between-group comparisons of continuous variables. Although the thickness measurements of the anterior digastric and right anterior temporalis muscles showed deviations from normality based on the Shapiro – Wilk test, parametric tests were retained, as independent samples t-tests are considered robust to moderate departures from normality, particularly when group sizes are balanced and no extreme outliers are present. Paired samples t-tests were applied to evaluate within-subject differences between clenching and resting conditions for the masseter and anterior temporalis muscles. Independent samples t-tests were also used to compare continuous variables between males and females, and between participants with and without TMD. Muscle asymmetry was assessed using the asymmetry index [31], calculated from paired left – right measurements. The Friedman test was used to compare asymmetry indices across different masticatory muscles within the same individuals, reflecting a repeated-measures structure rather than simple between-group comparisons.

To further investigate the influence of perceived stress, TMD severity, gender, and regular medication use on muscle thickness under resting and clenching conditions, linear regression models were constructed. Given the exploratory nature of the study and the limited sample size, formal correction for multiple comparisons (e.g., Bonferroni adjustment) was not applied, as such corrections may substantially increase the risk of Type II error and obscure potentially meaningful associations. Instead, findings were interpreted cautiously, with emphasis on effect sizes, consistency across analyses, and biological plausibility rather than sole reliance on *p*-values.

Among the models tested, those predicting the thickness of the right and left anterior digastric muscles demonstrated the greatest explanatory power. Before model construction, all potential confounders – including regular smoking, alcohol consumption, medication use, systemic conditions, age, gender, and TMD status (absence/presence) – were entered as candidate variables in a stepwise multiple linear regression procedure. Given the significantly higher prevalence of TMD in the high perceived stress group, TMD status was explicitly treated as a potential confounding variable rather than an intermediate outcome. Variable selection was performed using a stepwise procedure in order to explore which factors contributed most strongly to variability in anterior digastric muscle thickness. Given the relatively small sample size, this approach was considered exploratory and is acknowledged as potentially prone to model instability and overfitting. Accordingly, regression results were interpreted cautiously and viewed as hypothesis-generating rather than confirmatory. To assess the robustness of these findings, additional regression models were examined using pre-specified covariates entered simultaneously. These sensitivity analyses yielded comparable directional associations, supporting the consistency of the observed relationships. No stratified regression analyses by TMD subgroup were performed due to limited subgroup sizes, particularly within the moderate-to-severe TMD category. Based on these analyses, supplementary regression models were constructed to examine the independent associations of gender and perceived stress group (categorical) with anterior digastric muscle thickness. Taken together, the relatively small sample size, the number of statistical comparisons performed, and the exploratory use of regression modeling necessitate cautious interpretation of all findings, which should be regarded as descriptive and hypothesis-generating rather than definitive.

Results

A total of 40 participants, including 16 males and 24 females aged between 23 and 25 years (24.1 ± 1.65), were included in the study. Of these, 20 participants were classified as having normal perceived stress levels, whereas the remaining 20 were categorized as having high perceived stress levels. With respect to TMD, 25 participants presented with no signs or only mild symptoms, while 15 exhibited moderate to severe findings. As presented in Table 1, the prevalence of regular medication use and TMD was higher in the high perceived stress group compared with the normal stress group ($p = .028$ and $p = .020$, respectively). No statistically significant differences were observed in the remaining variables ($p > .05$).

The statistical comparisons of age, FAI scores, and muscle thicknesses between the normal and high perceived stress groups are presented in Table 2. Participants in the high perceived stress group

Table 1. Relationship between perceived stress levels and sociodemographic and health-related characteristics among participants.

	Participants with Normal Perceived Stress Level ($N = 20$) N (%)	Participants with High Perceived Stress Level ($N = 20$) N (%)	p-value
Gender			
Female	9 (45)	15 (75)	.053*
Male	11 (55)	5 (25)	
Mother's Educational Level			
No education/Primary school	5 (25)	5 (25)	.256**
Secondary/High school	13 (65)	9 (45)	
University/Doctoral degree	2 (10)	6 (30)	
Father's Educational Level			
No education/Primary school	8 (40)	5 (25)	.055**
Secondary/High school	8 (40)	3 (15)	
University/Doctoral degree	4 (20)	12 (60)	
Parental Monthly Income			
Below Average	6 (30)	10 (50)	.154*
Above Average	14 (70)	10 (50)	
Place of Residence			
House	13 (65)	10 (50)	.490*
Dormitory	7 (35)	10 (50)	
Regular Smoking			
No	15 (75)	15 (75)	1.000*
Yes	5 (25)	5 (25)	
Regular Alcohol Consumption			
No	12 (60)	10 (50)	.525*
Yes	8 (40)	10 (50)	
Regular Medication Use			
No	18 (90)	12 (60)	**.028***
Yes	2 (10)	8 (40)	
Presence of Diagnosed Disorder			
No	12 (60)	15 (75)	.311*
Yes	8 (40)	5 (25)	
Presence of TMD			
No	16 (80)	9 (45)	**.020***
Yes	4 (20)	11 (55)	

N: Number, TMD: Temporomandibular disorders. *Pearson Chi-square test, **Fisher exact test. Bold values mean statistically significant ($p < .05$).

demonstrated higher FAI scores compared with those in the normal stress group ($p = .035$). No differences were observed between the groups in the thicknesses of the left masseter or anterior temporalis muscles in either clenching or resting positions ($p > .05$). However, the thicknesses of the right and left anterior digastric muscles ($p = .008$ and $p = .005$, respectively), as well as the right masseter muscle during clenching ($p = .044$), were greater in the normal perceived stress group than in the high stress group. Notably, these patterns coincided with the higher prevalence of moderate-to-severe TMD observed in the high perceived stress group, suggesting that the observed muscle thickness differences may reflect overlapping distributions of stress and TMD severity. Representative ultrasonographic images of the masseter and anterior temporalis muscles in a participant with normal perceived stress are shown in Figure 2, illustrating marked muscle thickening during clenching compared with resting. Figure 3 depicts the corresponding images from a participant with high perceived stress, demonstrating relatively reduced muscle thickness under both conditions.

Table 3 presents the comparisons of PSS-14 and FAI scores, as well as muscle thicknesses, between female and male participants. Females demonstrated higher PSS-14 scores than males ($p = .033$). In contrast, males exhibited greater right masseter muscle thickness in both clenching ($p = .014$) and resting ($p = .019$) positions. The left masseter muscle was also thicker in males than in females during resting ($p = .041$). Furthermore, both right and left anterior digastric muscles were thicker in males compared with females ($p = .001$ and $p = .002$, respectively).

To facilitate interpretation of TMD-related findings, participants were categorized into two groups based on TMD presence and severity: those with no or mild findings ($n = 25$) and those with moderate or severe findings ($n = 15$). Comparisons of PSS-14 scores and muscle thicknesses between these groups are presented in Table 4. Participants with moderate or severe TMD demonstrated higher PSS-14 scores ($p = .005$). In contrast, the right anterior digastric muscle was thicker in the group with no or mild TMD ($p = .022$). This pattern mirrored the stress-group comparisons, further highlighting the close overlap between perceived stress levels and TMD severity in relation to anterior digastric muscle thickness. In addition to severity classification, DC/TMD clinical diagnoses were also recorded to

Table 2. Comparison of age, Fonseca Anamnestic index scores, and muscle thickness between normal and high perceived stress level groups.

	Participants with Normal Perceived Stress Level ($N = 20$) Mean±SD	Participants with High Perceived Stress Level ($N = 20$) Mean±SD	p-value*
Age	21.95 ± 1.82	22.25 ± 1.48	.571
Fonseca Anamnestic Index scores	35.5 ± 22.24	52.25 ± 25.98	**.035**
Right Masseter Muscle Thickness			
Clenching	13.52 ± 3.31	11.71 ± 2.05	**.044**
Resting	9.14 ± 2.39	8.08 ± 1.44	.096
Difference	4.38 ± 1.43	3.63 ± 1.32	.095
Left Masseter Muscle Thickness			
Clenching	12.40 ± 2.84	11.32 ± 2.16	.182
Resting	7.99 ± 2.09	7.20 ± 1.31	.160
Difference	4.41 ± 1.4	4.12 ± 1.49	.523
Right Anterior Temporalis Muscle Thickness			
Clenching	9.34 ± 2.32	9.05 ± 1.35	.627
Resting	8.53 ± 2.01	8.23 ± 1.18	.562
Difference	0.81 ± 0.63	0.82 ± 0.46	.842
Left Anterior Temporalis Muscle Thickness			
Clenching	10.23 ± 2.07	9.45 ± 1.99	.229
Resting	8.71 ± 1.78	8.53 ± 1.74	.748
Difference	1.52 ± 0.75	0.92 ± 0.52	**.032**
Right Anterior Digastric Muscle Thickness	6.76 ± 1.61	5.65 ± 0.61	**.008**
Left Anterior Digastric Muscle Thickness	6.91 ± 1.63	5.69 ± 0.76	**.005**

SD: Standard deviation. *Independent samples t-test. Bold values mean statistically significant ($p < .05$).

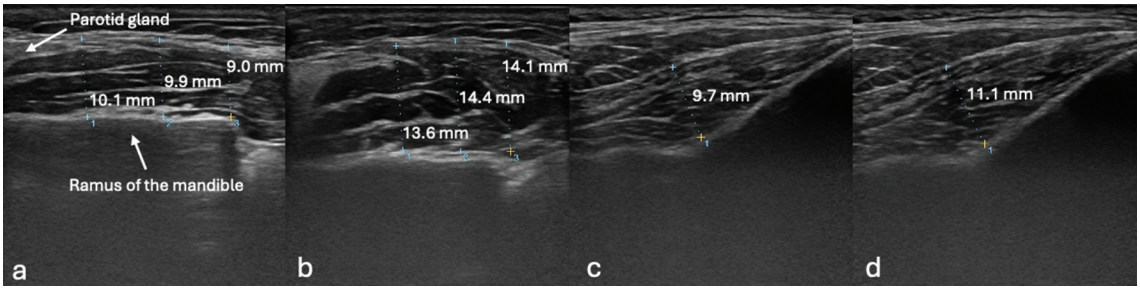


Figure 2. Ultrasonographic images of a participant with normal perceived stress level. Panels (a) and (b) show the masseter muscle measured at three standardized sites: the posterior region adjacent to the parotid gland, the central thickest point, and the anterior region of the mandibular ramus, in both resting and maximum voluntary clenching positions. Panels (c) and (d) display measurements of the anterior portion of the temporalis muscle during resting and clenching.

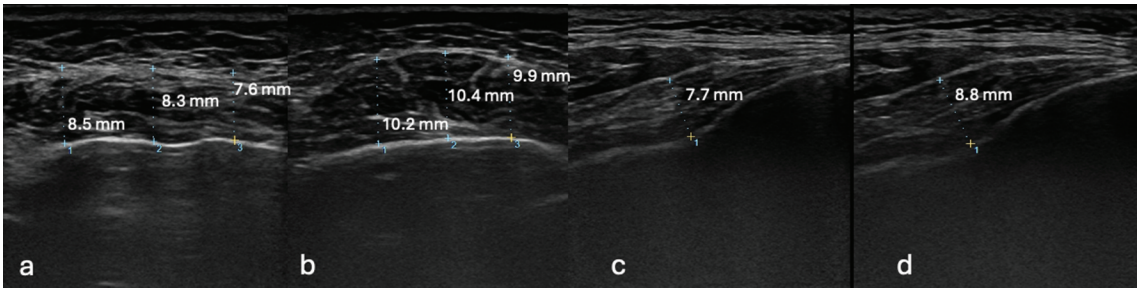


Figure 3. Ultrasonographic images of a participant with high perceived stress level. Panels (a) and (b) show the masseter muscle in resting and maximum voluntary clenching positions, respectively. Panels (c) and (d) display the anterior horizontal portion of the temporalis muscle in resting and clenching states.

Table 3. Comparison of Perceived Stress Scale and Fonseca Anamnestic Index scores and muscle thickness between genders.

	Male (N = 16) Mean±SD	Female (N = 24) Mean±SD	*p*-value*
Perceived Stress Scale Score	29.44 ± 6.97	34.67 ± 7.57	**.033**
Fonseca Anamnestic Index Score	36.88 ± 23.8	48.54 ± 25.73	.156
Right Masseter Muscle Thickness			
Clenching	13.95 ± 3.23	11.72 ± 2.25	**.014**
Resting	9.51 ± 2.3	8.01 ± 1.59	**.019**
Difference	4.44 ± 1.56	3.71 ± 1.4	**.006**
Left Masseter Muscle Thickness			
Clenching	12.67 ± 2.93	11.32 ± 2.16	.101
Resting	8.29 ± 2.21	7.13 ± 1.25	**.041**
Difference	4.38 ± 1.23	4.19 ± 1.31	.237
Right Anterior Temporalis Muscle Thickness			
Clenching	9.63 ± 2.24	8.9 ± 1.58	.240
Resting	8.7 ± 2.01	8.16 ± 1.33	.314
Difference	0.93 ± 0.65	0.74 ± 0.5	.545
Left Anterior Temporalis Muscle Thickness			
Clenching	10.32 ± 2.08	9.52 ± 2	.229
Resting	8.84 ± 1.9	8.46 ± 1.65	.504
Difference	1.48 ± 0.65	1.06 ± 0.68	.239
Right Anterior Digastric Muscle Thickness	7.15 ± 1.61	5.57 ± 0.49	**.001**
Left Anterior Digastric Muscle Thickness	7.26 ± 1.63	5.66 ± 0.72	**.002**

SD: Standard deviation. *Independent samples t-test. Bold values mean statistically significant ($p < .05$).

Table 4. Comparison of Perceived Stress Scale scores and muscle thickness among different groups based on the presence and severity of temporomandibular disorder.

	Absence of TMD/Mild TMD (N = 25) Mean±SD	Moderate TMD/Severe TMD (N = 15) Mean±SD	*p*-value*
Perceived Stress Scale Score	30 ± 7.88	36.87 ± 5.18	**.005**
Right Masseter Muscle Thickness			
Clenching	12.94 ± 3.16	12.05 ± 2.28	.348
Resting	8.98 ± 2.19	7.99 ± 1.57	.134
Difference	3.96 ± 1.43	4.06 ± 1.41	.827
Left Masseter Muscle Thickness			
Clenching	12.26 ± 2.66	11.19 ± 2.29	.206
Resting	7.78 ± 1.96	7.29 ± 1.41	.410
Difference	4.48 ± 1.41	3.9 ± 1.46	.221
Right Anterior Temporalis Muscle Thickness			
Clenching	9.33 ± 1.91	8.97 ± 1.87	.563
Resting	8.5 ± 1.71	8.17 ± 1.52	.534
Difference	0.83 ± 0.53	0.8 ± 0.58	.808
Left Anterior Temporalis Muscle Thickness			
Clenching	9.85 ± 1.71	9.81 ± 2.57	.955
Resting	8.66 ± 1.44	8.53 ± 2.21	.822
Difference	1.19 ± 0.58	1.08 ± 0.83	.631
Right Anterior Digastric Muscle Thickness	6.52 ± 1.52	5.67 ± 0.69	**.022**
Left Anterior Digastric Muscle Thickness	6.53 ± 1.59	5.91 ± 0.94	.175

TMD: Temporomandibular disorder, SD: Standard deviation. *Independent samples t-test. Bold values mean statistically significant ($p < .05$).

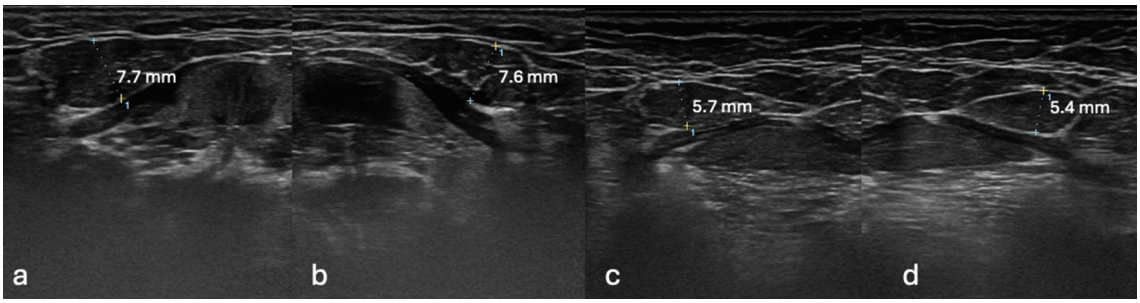


Figure 4. Ultrasonographic images demonstrating the anterior digastric muscle thickness in participants with normal and high perceived stress levels. Panels (a) and (b) illustrate the right and left anterior digastric muscles of a participant with normal stress level, while panels (c) and (d) present the corresponding sides of a participant with high stress level.

Table 5. Linear regression results for the effects of gender and perceived stress levels on right and left anterior digastric muscle thickness.

	Right Anterior Digastric Muscle Thickness			Left Anterior Digastric Muscle Thickness		
	B	β	*p*-value*	B	β	*p*-value*
Constant	7.369		**< .001**	7.513		**< .001**
Gender†	− 1.364	− 0.511	**< .001**	− 1.341	− 0.475	**.001**
Perceived Stress Level‡	− 0.701	− 0.268	**.048**	− 0.823	− 0.298	**.032**
R^2 (adjusted)	0.384			0.368		
F	13.174			12.378		

B: Unstandardized regression coefficient, β: Standardized regression coefficient, R^2: Determination coefficient, F: Overall model significance and variance explanation. †Reference: Male, ‡Reference: Normal perceived stress level. *Multiple linear regression analysis. Bold values mean statistically significant ($p < .05$).

characterize the sample. Among participants with moderate-to-severe TMD, 10 exhibited myofascial pain, 3 had localized myalgia, and 2 presented with intra-articular disorders. However, because the distribution of diagnostic subtypes was highly uneven, DC/TMD categories were not included in comparative subgroup analyses to avoid reduced statistical power and unstable comparisons. Exploratory comparisons of anterior digastric muscle thickness across DC/TMD diagnostic subtypes revealed no differences (myofascial pain: 5.2 ± 0.8 mm; localized myalgia: 5.4 ± 0.6 mm; intra-articular disorders: 5.0 ± 0.9 mm; $p > .05$). Given the small and unbalanced subgroup sizes, these findings should be interpreted cautiously. Figure 4 provides comparative ultrasonographic views of the anterior digastric muscles in participants with normal and high perceived stress levels, illustrating stress-related differences in muscle thickness.

The asymmetry index of all evaluated muscles was also compared between groups. According to the Friedman test, no differences in muscle asymmetry were observed between high and normal perceived stress groups, between males and females, or between participants with no/mild TMD and those with moderate/severe TMD ($p > .05$ for all comparisons). These results indicate that asymmetry indices did not vary meaningfully across stress, gender, or TMD subgroups in this sample.

Table 5 summarizes the effects of gender and perceived stress level on the thickness of the right and left anterior digastric muscles, as determined by multiple linear regression analysis. For the right anterior digastric muscle, both female gender and high perceived stress level were associated with reduced muscle thickness ($p < .001$ and $p = .048$, respectively). The regression model explained a moderate proportion of variance ($F = 13.174$, adjusted $R^2 = 0.384$). Similarly, for the left anterior digastric muscle, both female gender and high perceived stress level were associated with reduced thickness ($p = .001$ and $p = .032$, respectively; $F = 12.378$, adjusted $R^2 = 0.368$). Although perceived stress remained associated with anterior digastric thickness in these models, the findings should be interpreted in the context of the concurrent distribution of TMD severity across stress groups.

Discussion

This study examined the association between perceived stress, gender, and TMD status and ultrasonographically assessed masticatory muscle thickness. Differences were observed in the thickness of the right and left anterior digastric muscles between individuals with normal and high perceived stress levels. In addition, anterior digastric muscle thickness varied according to TMD severity, and both the masseter and anterior digastric muscles showed gender-related differences. Multiple linear regression analyses indicated that perceived stress level and gender were independently associated with anterior digastric muscle thickness; however, the explanatory power of these models was low-to-moderate. Importantly, given the higher prevalence of TMD in the high perceived stress group, the observed associations must be interpreted cautiously, as they may partially reflect TMD-related neuromuscular adaptations rather than effects attributable solely to perceived stress. Accordingly, the findings support an association-based relationship between perceived stress and specific masticatory muscle characteristics rather than a direct causal effect, and the null hypothesis was rejected within this exploratory and correlational framework.

A review of the literature revealed no prior studies evaluating the association between perceived stress and masticatory muscle thickness using ultrasonography, highlighting the novelty of the present study, particularly in young adult populations. Zielinski et al. [45] evaluated the relationship between stress and masticatory muscle activity using EMG in combination with the PSS-14 and reported no significant association between stress and activity in the masseter or anterior temporalis muscles. However, they observed asymmetry in muscle

activation patterns, suggesting that stress-related neuromuscular modulation may manifest as functional imbalance rather than uniform changes in muscle activity. Their study did not include assessment of the digastric muscle, limiting direct comparison with the present findings. In contrast, the current study identified an association between perceived stress levels and anterior digastric muscle thickness. This difference should be interpreted cautiously, as ultrasonographic thickness reflects morphological characteristics rather than functional activation, and the observed association may represent an indirect or adaptive neuromuscular response rather than a direct effect of stress. Moreover, asymmetry findings – although not statistically significant between groups – suggest that stress-related influences on the stomatognathic system, if present, are subtle and modulated by coexisting factors such as TMD status and individual motor control patterns.

Owczarek et al. [46, 47] reported increased masseter muscle activity in dental students experiencing higher psychosocial stress, suggesting enhanced neuromuscular activation rather than structural hypertrophy. In contrast, the present study observed lower right masseter thickness during clenching in the high-stress group, a finding that appears inconsistent with EMG-based literature. This discrepancy may be explained by methodological differences. Ultrasonographic thickness reflects static anatomical dimensions and cannot differentiate between reduced activation, transient muscle inhibition, connective tissue changes, or true atrophy. Moreover, cross-sectional imaging does not capture tonic hyperactivity or cumulative functional loading over time. Accordingly, the reduced masseter thickness observed in the high-stress group – limited to one side – should not be interpreted as evidence of diminished function, but rather as a morphological observation whose functional significance remains uncertain.

Findings from a longitudinal pilot study [48] indicated that exam-related stress in physiotherapy students was associated with decreased masticatory muscle contraction and contributed to the progression of TMD. In that study, sustained reductions in functional muscle activity may plausibly be accompanied by morphological changes, such as decreased muscle thickness. This observation is partially consistent with the present findings, in which participants with high perceived stress exhibited reduced bilateral anterior digastric thickness. Notably, however, only the right anterior digastric muscle differed between TMD groups, suggesting that stress-related and TMD-related effects may overlap but are not interchangeable.

In another prospective study, shear wave elastography revealed reduced masseter stiffness following manual therapy and occlusal splint use, yet no association with perceived stress scores was observed [49]. Overall, existing studies report heterogeneous findings regarding the relationship between stress and masticatory muscle characteristics. Differences in assessment methods – including EMG, elastography, and ultrasonography – as well as study design and outcome definitions likely contribute to these inconsistencies. Unlike prior investigations focusing on muscle activity or stiffness, the present study assessed muscle thickness using ultrasonography, a modality that provides static morphological information but does not capture neuromuscular activation or functional load. Consequently, the observed associations – particularly for the anterior digastric muscle – should be interpreted as indirect and exploratory.

Although the present study categorized participants based on overall TMD severity, exploratory analysis of DC/TMD diagnostic subtypes revealed no significant differences in anterior digastric muscle thickness between myofascial pain, localized myalgia, and intra-articular disorder groups. This finding should be interpreted cautiously due to the small and unbalanced subgroup sizes, which limit statistical power and preclude definitive conclusions. Nevertheless, the observation suggests that reduced digastric thickness may represent a general feature associated with TMD-related dysfunction rather than a subtype-specific adaptation.

Beyond these findings, accumulating evidence underscores the complex and bidirectional relationship between psychological burden, TMD, and stomatognathic muscle function. Psychological stress, autonomic dysregulation, pain perception, and TMD-related symptoms frequently co-occur, making it challenging to disentangle their independent effects on muscle morphology. Experimental studies have shown that acute psychological or visceral stress can transiently increase masseter muscle activity and discomfort, suggesting that emotional arousal may modulate masticatory muscle behavior through autonomic pathways [13]. Importantly, individuals exposed to higher psychological stress often present with increased TMD prevalence, greater pain burden, and higher rates of medication use – each of which may independently influence muscle activation patterns, protective motor adaptations, and measured muscle thickness. Clinical studies have demonstrated that bruxism and TMD populations frequently exhibit elevated anxiety, stress sensitivity, somatization, and pain-related disability [18, 50]. Taken together, the present findings indicate that higher perceived stress in this cohort may function primarily as a marker of a broader clinical phenotype characterized by increased TMD prevalence, pain-related adaptation,

and potentially altered neuromuscular behavior, rather than as an independent driver of morphological change. In this context, differences in anterior digastric muscle thickness likely reflect composite influences of TMD-related pain adaptation, medication use, and behavioral factors, rather than a stress-specific effect.

In the present study, participants with high perceived stress levels exhibited reduced anterior digastric muscles thickness. This finding points to a possible association between chronic psychological burden and morphological variation in the suprahyoid musculature. Unlike the masseter and anterior temporalis muscles, the digastric muscle functions primarily as a low-load stabilizing muscle involved in mandibular depression and postural control rather than forceful clenching [51]. Alterations in its thickness may therefore reflect changes in neuromuscular coordination or pain-related motor adaptation rather than a direct effect of perceived stress [52, 53]. These interpretations remain speculative and cannot be substantiated without longitudinal data or concurrent functional assessments such as EMG. Given the strong co-occurrence of perceived stress and TMD in the present sample, as well as the absence of bruxism assessment, the observed differences in digastric muscle thickness cannot be attributed to perceived stress per se. Instead, the signal observed in this study likely reflects the combined influence of TMD-related pain, altered motor control strategies, medication use, and other behavioral factors.

Although sociodemographic and health-related variables were included within the study objectives, most of these factors did not demonstrate meaningful associations with muscle thickness or perceived stress in the advanced analyses. This is likely related to the homogeneity of the cohort. Gender emerged as the only sociodemographic variable associated with anterior digastric thickness, with females exhibiting lower values than males, consistent with established anatomical and physiological differences reported in prior ultrasonographic studies [54, 55].

Previous ultrasonographic studies have demonstrated that muscle morphology and mechanical properties can be reliably assessed in various stomatognathic conditions. For instance, masseter muscle thickness and elasticity have been shown to differ significantly between bruxism patients and controls, and to change following therapeutic interventions such as splint therapy [56, 57]. Similarly, ultrasonographic evaluation of the masticatory muscles has revealed meaningful associations with pain levels, symptom duration, and functional parameters in patients with myofascial pain disorder [58]. Moreover, recent investigations have expanded the application of ultrasonography to the assessment of awake bruxism behaviors, showing that muscle thickness and functional activation patterns can be objectively quantified, and that ultrasonography-derived parameters may complement behavioral or self-reported measures of parafunctional activity [10, 59, 60]. These findings support the utility of ultrasonography as an objective, non-invasive method for investigating structural and functional adaptations within the masticatory system [24, 25, 27].

From a clinical perspective, the anterior digastric muscle may warrant consideration within a broader biopsychosocial framework of masticatory system assessment. Reduced thickness in this muscle may reflect altered neuromuscular balance between agonist and antagonist muscle groups or pain-related motor adaptation. Such interpretations are aligned with current IADR/INFORM recommendations, which emphasize that TMDs should be understood through an integrated biopsychosocial model rather than isolated structural findings [61]. However, ultrasonographic muscle thickness represents a static anatomical parameter and should not be interpreted as a diagnostic marker of stress exposure, TMD severity, or treatment response. At this stage, ultrasonography of the anterior digastric muscle should be regarded strictly as an adjunctive descriptive tool rather than a clinical biomarker.

One of the main strengths of the present study is its novel application of ultrasonographic imaging to investigate the association between perceived stress levels and anterior digastric muscle thickness – an area not previously addressed in the literature. The integration of standardized instruments such as the PSS-14 and FAI with objective ultrasonographic measurements enhances the methodological rigor of the study. Furthermore, both the TMJ examinations and muscle thickness assessments were conducted by calibrated, experienced examiners with high intra-rater reliability scores, thereby contributing to the consistency and reliability of the data. The use of linear regression analysis to identify independent predictors of muscle thickness, including stress and gender, represents an additional strength, as it provides a more comprehensive understanding of the variables involved. Although the present study focused primarily on the anterior digastric muscle due to its significant association with perceived stress in the analyses, masseter and temporalis measurements were also evaluated. These muscles did not demonstrate meaningful differences between stress groups, which explains their comparatively limited emphasis in the discussion. This aligns with the

regression results, where stress and gender emerged as predictors only for the anterior digastric muscle.

Despite these strengths, several limitations should be acknowledged. The relatively small sample size and cross-sectional design limit the generalizability of the findings and preclude causal interpretations. Although multiple comparisons and subgroup analyses were necessary to explore associations across different muscles and conditions, the limited sample size increases the risk of Type I error. The inclusion of only dental students from a single institution resulted in a highly homogeneous study population. While this homogeneity was intentionally selected to reduce environmental and occupational variability and to strengthen internal validity, it inevitably limits external validity and restricts the generalizability of the findings to broader or more diverse populations. Moreover, although validated instruments were employed, perceived stress remains a subjective measure and may fluctuate over time.

While ultrasonography is a reliable and widely used tool for assessing masticatory muscle morphology, muscle thickness measurements obtained from cross-sectional ultrasonography cannot distinguish between reduced muscle activation, true muscular atrophy, connective tissue infiltration, or transient changes related to hydration status or tissue compressibility. Therefore, reduced thickness should not be interpreted as definitive evidence of muscle atrophy or altered functional load. In addition, although a standardized imaging protocol and high intra-examiner reliability were achieved, ultrasonography does not capture dynamic muscle activity or neuromuscular recruitment patterns, which limits functional interpretation. Another important limitation is the absence of direct assessment of awake or sleep bruxism. Although bruxism is widely recognized as a major determinant of masticatory muscle loading and hypertrophy, it was not formally evaluated using validated diagnostic criteria or instrumental recordings. Consequently, residual confounding related to parafunctional activity cannot be excluded, and the present findings should be interpreted within this context.

The regression models demonstrated weak-to-moderate explanatory power, supporting the interpretation of the results as hypothesis-generating associations rather than strong predictive evidence. Future studies combining ultrasonography with longitudinal designs, EMG, elastography, or functional imaging techniques are warranted to better differentiate morphological from functional adaptations and to clarify the clinical relevance of stress-related changes in masticatory muscles.

Conclusion

The present study demonstrated an association between perceived stress levels and ultrasonographically measured anterior digastric muscle thickness in young adults. Participants with higher perceived stress exhibited reduced digastric thickness, and gender and stress level emerged as factors associated with this parameter. However, given the cross-sectional design, the homogeneous study population, and the presence of important confounders – particularly temporomandibular disorder status, pain-related adaptation, and parafunctional behaviors – these findings should be interpreted with caution.

The observed differences should be regarded primarily as a descriptive anatomical signal. Ultrasonographic muscle thickness reflects a static morphological characteristic and, on its own, does not provide diagnostic, prognostic, or therapeutic guidance for stress-related or temporomandibular disorders in routine clinical practice.

From a research perspective, the findings suggest that the anterior digastric muscle may represent a potentially informative structure to consider in future multimodal and longitudinal investigations. Integrating ultrasonography with functional assessments (such as electromyography or elastography), behavioral evaluation of parafunctional activity, and longitudinal symptom tracking in populations with overlapping stress and temporomandibular disorders phenotypes may help to further elucidate the underlying mechanisms and contextual relevance of the observed associations.

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ORCID

Berkant Sezer DDS, MSc http://orcid.org/0000-0001-9731-6156
Gamze Coşan Ata DDS, MSc http://orcid.org/0000-0003-4926-4405

Data availability statement

The data that support the findings of this study are available from the corresponding author (B.S.) upon reasonable requests.

Author contributions

Conceptualization: [Berkant Sezer], [Aysu Şahin], [Doğa Doğan], [Toprak Efe Ersöz]; Methodology: [Berkant Sezer], [Gamze Coşan Ata]; Formal analysis and investigation: [Berkant Sezer], [Gamze Coşan Ata], [Aysu Şahin], [Doğa Doğan], [Toprak Efe Ersöz]; Writing – original draft preparation: [Berkant Sezer]; Writing – review and editing: [Berkant Sezer], [Gamze Coşan Ata]; Supervision: [Berkant Sezer].

Ethical approval

Ethical approval for this cross-sectional observational study was obtained from the Non-Interventional Clinical Research Ethics Committee of Çanakkale Onsekiz Mart University on May 8 2024 (protocol no. 02/2024–14). All procedures adhered to the ethical principles outlined in the Declaration of Helsinki.

Informed consent

Prior to participation, all potential participants received detailed oral and written information regarding the study, and written informed consent was obtained. The consent form included comprehensive explanations to ensure participants fully understood the nature and scope of the research.

Consent to participate

Written informed consent was obtained from all participants. Participation was entirely voluntary.

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