



Factors associated with masseter muscle quality assessed from ultrasonography in community-dwelling elderly individuals: A cross-sectional study



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ABSTRACT

Background: Associations between masseter muscle thickness (MMT) and limb muscle thickness, and between grip strength and MMT, as well as tooth-loss, have been reported previously. The previous study also showed that masseter muscle mass could be a better marker of sarcopenia than psoas muscle mass. Although the association between MMT and muscle strength is also known, the quality of the masseter muscle were not assessed in detail previously. We examined the relationship of masseter muscle echo intensity (MMEI) with skeletal muscle, physical function, and nutrition status, in order to determine whether MMEI could be a good indicator of these parameters.

Methods: We assessed 139 community-dwelling elderly individuals (men: 65, women: 74). Age, body mass index (BMI), skeletal muscle mass index, grip strength, walking speed, calf circumference, tooth-loss (Eichner classification), occlusal force, MMT, and MMEI were obtained. In multiple regression analysis, MMEI were set as dependent variables.

Results: Multiple regression analysis revealed BMI ($p < 0.05$), grip strength ($p < 0.01$), walking speed ($p < 0.01$), and MMT ($p < 0.01$) as factors with significant association with MMEI.

Conclusions: MMT is related to occlusal force and MMEI. MMEI was related strongly to grip strength and walking speed, but not to tooth-loss. However, MMEI, which is easily determined ultrasonographically, could be a good indicator of grip strength and walking speed, and thus may be predictive of dynapenia.

1. Introduction

The masseter muscle is a masticatory muscle, and masseter muscle thickness (MMT) attenuates with tooth-loss. The MMT of edentulous patients is reported to be significantly thinner than that of dentate patients (Bhoyar, Godbole, Thombare, & Pakhan, 2012). Dental prosthodontics, such as implants and dentures increase MMT (Gonçalves, Campos, Gonçalves, de Moraes, & Rodrigues Garcia, 2013). Previous studies have also revealed a relationship between MMT and occlusal force (Raadsheer, van Eijden, van Ginkel, & Prah-Andersen, 1999) as well as chewing ability (Müller et al., 2012); thus, the masseter muscle is a key player in oral ingestion.

MMT and limb muscle thickness are also reportedly related (Raadsheer, Van Eijden, Van Ginkel, & Prah-Andersen, 2004).

Additionally, we previously examined the relationship of tooth-loss, aging, skeletal muscle mass, and muscle strength with MMT in community-dwelling individuals older than 65 years, and reported an association of tooth-loss and grip strength with MMT (Yamaguchi et al., 2018). The previous study also showed the association between MMT and SMI (Umeki et al., 2018). In addition, in a 2-year longitudinal study on trauma in elderly patients, it has been shown that masseter muscle mass can be a better marker of sarcopenia than psoas muscle mass, which is the golden standard of muscle mass measurement (Wallace et al., 2017). These studies showed that masseter muscle is strongly related to both oral function and limb skeletal muscle.

Few other reports have used the same approach for determining the quality of the masseter muscle that we used in our previous study of MMT. Therefore, it is unknown whether masseter muscle echo intensity

Abbreviations: BMI, body mass index; CC, calf circumference; MMEI, masseter muscle echo intensity; MMT, masseter muscle thickness; SMI, skeletal muscle mass index

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(MMEI), similar to MMT, is related to occlusal force and whole body skeletal muscle. Muscle quality is expressed by echo intensity on diagnostic ultrasonic imaging (Young, Jenkins, Zhao, & Mccully, 2015). Echo intensity represents the fat and fibrous tissue in muscle. The previous study showed an association between echo intensity and physical function (Rech et al., 2014), it has shown that echo intensity can be used for diagnosis of sarcopenia (Yamada et al., 2017). The relationship of muscle quantity and quality with limb muscle strength has been reported previously (Fukumoto et al., 2012), but no similar study of the masticatory muscles, such as the masseter muscle, has been reported. There has been only limited research in this context, such as a comparison of MMEI between patients with different forms of muscular dystrophy (DM) (Tieleman et al., 2012). In that study, the thickness and echo intensity of the masseter muscle, elbow flexors, forearm flexors, rectus femoris, and tibialis anterior of patients with DM1 and DM2 were compared; however, the association of MMEI with muscle strength and physical function has not been studied. Furthermore, there has been no research about MMEI in community-dwelling elderly individuals.

Therefore, the purpose of this research was two-fold. First, to examine the relationship of tooth-loss, skeletal muscle mass, and physical function with MMEI by using already reported research data (Yamaguchi et al., 2018) as well as new data, and to investigate whether MMEI could be an indicator of skeletal muscle mass and physical function, similar to MMT. Second, we aimed to examine whether MMEI is related to muscle strength in terms of occlusal force, as it is to limb muscle.

2. Materials and methods

2.1. Participants

Study participants were 139 healthy community-dwelling elderly individuals. Recruitment took place from December 2015 until August 2016, as follows. First, we recruited volunteers who participated in a health survey jointly conducted by Tokyo Medical and Dental University and Oyama city (Tochigi, Japan). Second, we recruited healthy elderly people who presented to the Gerodontology Clinic of the Dental Hospital at Tokyo Medical and Dental University for dental treatment. Third, we recruited healthy elderly people who attended the healthcare center in Kameari (Tokyo, Japan). We included healthy individuals who consented to participate in this study, who were self-reliant in terms of the activities of daily living, could follow instructions, and were ≥ 65 years old, and excluded individuals with a history of conditions that affect muscle mass, such as neuromuscular diseases, and those wearing pacemakers. Two of 177 consenting subjects were excluded because they fulfilled the exclusion criteria, and 20 were

excluded because they were less than 65 years old and thus did not meet the inclusion criteria. Of the 155 remaining subjects, 139 subjects for whom all required data were available, were finally included in analysis.

This study was conducted under the approval of Tokyo Medical and Dental University Ethics Committee (ref: D2014-047). Written informed consent was obtained from subjects for participation in the study.

2.2. Measurement of nutritional status, skeletal muscle mass, and physical function

Participants' height and weight were measured, and body mass index (BMI) was calculated as weight divided by the square of height. The calf circumference (CC) was measured in a sitting position, using a non-elastic tape measure. Skeletal muscle mass index (SMI) was obtained by bioelectrical impedance analysis using the Inbody S10 body composition analyzer (Inbody Japan, Inc., Tokyo, Japan). Measurements were taken with the subjects in a relaxed state in a sitting position, and the value obtained by dividing the appendicular muscle mass by the square of height was taken as the SMI. Grip strength was measured twice for the dominant hand; the higher value was used for analysis. To measure walking speed, we set a course consisting of a 2-m acceleration section, a 4-m walking section, and a 2-m deceleration section. When we set out the 5-m walking section, a 3-m acceleration and deceleration section were set. Walking speed was calculated based on the time required to complete the walking section at the subject's usual pace.

2.3. Measurement of oral condition

Tooth-loss was recorded by three dentists with more than 5 years of experience. The state of tooth-loss was grouped using Eichner classification (Eichner, 1955). The occlusal force was recorded at the first molar of natural teeth or dentures, with participants in a sitting position, using an Occlusal Force Meter GM10 (Nagano Keiki Co., Ltd., Tokyo, Japan) (Hirao et al., 2014). For subjects lacking first molars, occlusal force was determined in the vicinity of the first molar. Measurements were performed twice on the right side, and the maximum value was taken for analysis.

Masseter muscles were evaluated with an ultrasonic diagnostic apparatus (Mysono U6, Samsung Madison, Inc., Seoul, Korea), by a single dentist. Participants were in a sitting position, and the masseter muscle on the right side was measured at rest and during contraction. The probe was a linear type, with a broadband frequency of 5–12 MHz, and the gain was fixed at 45 dB for all measurements. The probe was placed parallel to the mandibular margin, approximately midway between the

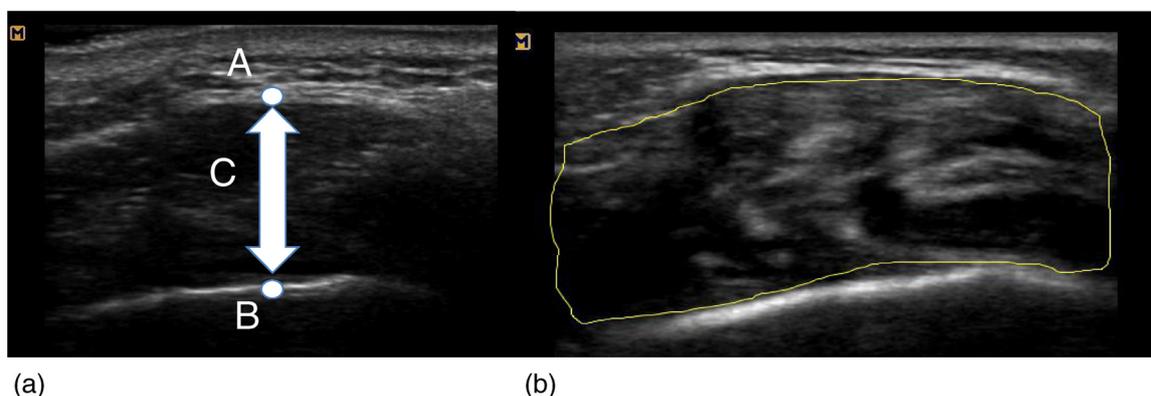


Fig. 1. Masseter muscle evaluation by ultrasound diagnostic apparatus.

(a) Masseter muscle thickness measurement.

(b) Masseter muscle echo intensity measurement.

(A) Masseter muscle surface (B) Mandibular ramus (C) Masseter muscle thickness.

zygomatic arch and the mandibular angle, perpendicular to the skin surface (Serra, Duarte Gavião, & dos Santos Uchôa, 2008). MMT was determined by the apparatus at the thickest part in the image (Fig. 1). The mean of two measurements was taken for analysis.

In the echo intensity analysis, a region of interest (ROI) in the image was determined so as to contain the maximum amount of muscle without bone and fascia, using ImageJ v.1.49 (National Institutes of Health, Bethesda, MD) (Fig. 1) (Fukumoto et al., 2012). The echo intensity was displayed as a number between 0 and 256 (values close to 0 were darker, and values close to 256 were lighter). The mean of two measurements was used for analysis. For MMEI analysis, the intra-class correlation coefficient (ICC) and the intra-rater reliability were determined. The ICC for determining the average measures twice was 0.9 for MMEI at rest and 0.87 for MMEI during contraction, both of which indicated high intra-rater reliability.

2.4. Statistical analyses

The sample size was calculated beforehand using G*Power3.1 software (Kiel University, Kiel, Germany). With an α of 0.05, power of 0.9, and an effect size of 0.24, based on a previous study (Yamaguchi et al., 2018), the required sample size was calculated to be 96 people.

Normal data distribution was confirmed using the Shapiro–Wilk test for each measurement item in the Eichner A, B, and C group. One-way analysis of variance, Kruskal–Wallis tests, and chi-square tests were used to investigate the significance of differences among Eichner groups. Correlations of MMEI with the respective factors were examined by Pearson's correlation coefficient and Spearman's rank correlation coefficient. Multiple regression analysis was performed with MMEI during contraction as dependent variables, and age, sex, tooth-loss, BMI, CC, SMI, grip strength, walking speed, MMT during contraction as independent variables. Tooth-loss was included in the regression model as a categorical variable (Eichner A, B, or C). For statistical analysis, Windows Japanese version SPSS 21 J (IBM Inc., Tokyo, Japan) was used.

3. Results

Subject characteristics are shown in Table 1. There were significant differences in age ($p < 0.01$), SMI ($p < 0.05$), walking speed ($p < 0.05$), occlusal force ($p < 0.01$), and MMEI ($p < 0.05$) among the groups. Table 2 shows the correlation matrix of the correlation of MMEI with each variable. No variables had $r > 0.7$.

Table 3 show the results of multiple regression analysis with MMEI as dependent variable. Age and sex were not identified as significant

factors in the multiple regression model. BMI ($p < 0.05$), grip strength ($p < 0.01$), walking speed ($p < 0.01$), and MMT ($p < 0.01$) were identified as significant factors. R was 0.59 and the adjusted R^2 was 0.3. The standard partial regression coefficient was -0.21 in BMI, -0.32 in grip strength, -0.25 in walking speed, -0.31 in MMT.

4. Discussion

In the present study, MMEI was not related to sex and age; additionally, despite involving a masticatory muscle, this parameter did not show association with tooth-loss in individuals with sufficient oral intake. Thus, MMEI may be more important in terms of the regular use of the masseter muscle for oral ingestion of food, rather than in terms of tooth-loss.

Previous studies have already reported the relationship of grip strength and SMI with MMT, showing a relationship between sarcopenia and masseter muscle mass. In this study, we showed the relation of MMEI to MMT, BMI, grip strength, and walking speed. In another cross-sectional study of healthy community-dwelling elderly individuals, grip strength correlated strongly with body skeletal muscle mass, rather than with knee extension strength, and was a relatively good indicator of muscle strength (Yamada et al., 2013). A study that tracked 4588 healthy subjects for up to 6 years reported that the prediction accuracy for reduced activities of daily living and gait disturbance did not change markedly over the follow-up period, irrespective of whether multiple physical function tests or only a walking speed test were used. Therefore, walking speed is considered to be an effective predictor of the motor function of the lower limbs (Guralnik et al., 2000). The present study showed that MMEI could be a good indicator of walking speed, as well as of grip strength, in addition to MMT, which is related to chewing ability.

Therefore, MMEI was related to systemic skeletal muscle, similar to MMT. Hormones such as IGF-1 and testosterone can be considered as a common cause of muscle deterioration in masticatory muscle and whole body skeletal muscle (Iinuma et al., 2012). These hormones are essential for muscle repair and augmentation, and their levels decrease with age (Albani et al., 2009). Masseter muscle mass has been shown to be strongly related to testosterone levels. A previous study in mice reported that masseter muscle mass deteriorated with castration, and increased by 38% with testosterone injection (Widmer & Morris-Wiman, 2010). This response was more robust than that seen in limb muscles. Since the association between MMT and MMEI was shown in this study, it can be inferred that these hormones are related to MMEI as they are to MMT and whole body skeletal muscle.

Ultrasonography is a relatively simple and non-invasive approach.

Table 1
Subjects' characteristics (N = 139).

	Eichner A n = 54	Eichner B n = 62	Eichner C n = 23	P-value
Age, years, median (IQR)	71.0 (7)	75.0 (10)	78.0 (10)	< 0.01 ^a
Sex, male, (%)	19 (35.2)	33 (53.2)	13 (56.5)	0.09 ^b
SMI, kg/m ² , mean \pm SD	6.5 \pm 1.1	7.1 \pm 1.0	7.0 \pm 1.0	< 0.05 ^c
BMI, kg/m ² , median (IQR)	22.6 (4.9)	23.5 (3.8)	23.7 (2.9)	0.5 ^a
CC, cm, median (IQR)	34.1 (4.2)	34.6 (3.7)	33.7 (3.0)	0.44 ^a
Grip strength, kg, median (IQR)	24.1 (10.8)	25.3 (8.2)	22.3 (16.6)	0.43 ^a
Walking speed, m/s, median (IQR)	1.4 (0.4)	1.2 (0.5)	1.1 (0.6)	< 0.05 ^a
Occlusal force, N, median (IQR)	302.5 (275.5)	158.0 (227.5)	77.0 (120.0)	< 0.01 ^a
MMT, mm, median (IQR)	12.2 (2.8)	11.6 (3.9)	11.0 (3.1)	0.1 ^a
MMEI at rest, median (IQR)	36.2 (19.7)	36.6 (18.8)	44.4 (19.7)	0.05 ^a
MMEI during contraction, median (IQR)	27.5 (17.0)	26.2 (14.7)	32.4 (13.6)	< 0.05 ^a

^aKruskal–Wallis test, ^bChi-square test; ^cOne-way analysis of variance.

SD: Standard deviation; IQR: Interquartile range; SMI: Skeletal muscle mass index; BMI: Body mass index; CC: Calf circumference; MMT: Masseter muscle thickness; MMEI: Masseter muscle echo intensity.

Table 2
Correlation matrix table of parameters.

	Sex	Age	BMI	Grip strength	Walking speed	SMI	CC	Tooth loss	Occlusal force	MMT	MMEI
Sex	1	-0.22*	-0.01	-0.59*	0.13	-0.62*	-0.12	-0.18*	-0.13	-0.35*	0.18
Age		1	-0.11	-0.20*	-0.27*	-0.01	-0.30*	0.37*	-0.16	-0.09	0.24*
BMI			1	0.11	-0.18*	0.46*	0.67*	0.07	-0.15	0.11	-0.17
Grip strength				1	0.28*	0.58*	0.42*	-0.03	0.21*	0.22*	-0.42*
Walking speed					1	-0.01	0.16	-0.24*	0.29*	-0.12	-0.27*
SMI						1	0.52*	0.19*	0.09	0.29*	-0.25*
CC							1	-0.14	0.10	0.15	-0.28*
Tooth loss								1	-0.45*	-0.16	0.17*
Occlusal force									1	0.34*	-0.23*
MMT										1	-0.39*
MMEI											1

* $p < .05$.

BMI: Body mass index; SMI: Skeletal muscle mass index; CC: Calf circumference; MMT: Masseter muscle thickness; MMEI: Masseter muscle echo intensity.

Since the masseter muscle is highly accessible, it is easily measured ultrasonographically, even in bedridden individuals, and patients are not required to change posture or remove clothes. Moreover, it has been reported that weakness of the lower muscle occurs before the loss of muscle mass in elderly people (Goodpaster et al., 2006). Weakened muscles and decreased motor function are included in the diagnosis of sarcopenia (Chen et al., 2014), and have also been proposed as markers of dynapenia (Manini & Clark, 2012). Low muscle strength in elderly people could also be a risk factor for mortality and disability (Newman et al., 2006). MMEI measurement could thus be used to prevent sarcopenia, and may be an indicator of dynapenia.

In this study, MMEI was not associated with occlusal force in healthy community-dwelling elderly individuals, which contradicted the findings of studies of the relationships of limb muscle echo intensity to date. This may be due to the difference in the muscle fibers constituting the masseter muscle and limb muscle, and differences in muscle atrophy. Muscle atrophy has various causes, such as aging and disuse: it has been reported that muscle mass decreased by 10% after 1 month of bed-rest (Berry, Berry, & Manelfe, 1993).

Muscle disuse can be one of the main causes of muscle atrophy in elderly people. Muscle atrophy due to disuse tends to occur mainly in type 1 fibers (Ciciliot, Rossi, Dyar, Blaauw, & Schiaffino, 2013), while atrophy due to aging predominantly occurs in type 2 fibers (Lexell, Taylor, & Sjöström, 1988). Both type 1 and type 2 muscle fibers are present in the masseter muscle and the biceps brachii, but type 1 fibers predominate in the masseter muscle, while type 2 fibers predominate in the biceps brachii (Monemi, Eriksson, Eriksson, & Thornell, 1998). Thus, muscle atrophy of the biceps brachii muscle tends to be caused by aging, while that of the masseter muscle is more likely to be caused by disuse. Typically, if a person ingests food orally, masseter muscle atrophy will not proceed rapidly. In addition, skeletal muscle has high

regenerative ability, mostly due to muscle satellite cells (Hawke & Garry, 2001). The number of muscle satellite cells in type 2 fibers, but not type 1 fibers, decreases with aging (Verdijk et al., 2007). Thus, the masseter muscle, which mainly comprises type 1 fibers, tends to maintain regenerative ability. In other words, muscle atrophy due to aging is less likely to occur in masticatory muscle than in limb muscle. Even if type 2 fibers decrease and the proportion of type 1 fibers increases with aging (Cullins & Connor, 2017), the masseter muscle will not change markedly, as type 1 fibers were already predominant.

We observed no association of age with MMEI in this study. Moreover, the association of MMEI with muscle strength was relatively weak, because atrophy due to aging was less than that of limb muscle. Continuous dental treatment could maintain the amount of masseter muscle activity (Von der Gracht, Derks, Haselhuhn, & Wolfart, 2017). Improvement of MMT and occlusal force by dental treatment has been reported in a previous study (Gonçalves et al., 2013). Teeth or dentures are necessary to maintain occlusal force; in addition, dental treatment affects MMT by preventing disuse of the masseter muscle. Moreover, we found a strong association of MMEI with MMT, suggesting that the improvement of MMT by dental treatment may have a good influence on MMEI.

This study had some limitations. Firstly, since the subjects were healthy community-dwelling individuals, our participants had relatively good nutritional status and whole body skeletal muscle mass. Secondly, as this was a cross-sectional study, our results cannot be used to show a causal relationship. In future, we plan to perform more detailed research on patients with dysphagia or elderly individuals showing some deterioration.

In this study, there was no relation of tooth-loss and occlusal force with MMEI in healthy community-dwelling elderly individuals, but grip strength and walking speed were strongly related to MMEI. MMEI could

Table 3
Multiple regression analysis values with MMEI during contraction as the dependent variable.

	Standard partial regression coefficient	P-value	95% CI	R	Adjusted R ²
Age	0.02	0.85	-0.29 to 0.35	0.59	0.30
Sex	-0.01	0.95	-6.16 to 5.76		
BMI	-0.21	< .05	-1.51 to -0.03		
SMI	0.10	0.46	-1.84 to 4.03		
CC	0.01	0.93	-0.65 to 0.72		
Grip strength	-0.32	< .01	-0.84 to 0.16		
Walking speed	-0.25	< .01	-13.11 to -2.73		
Tooth loss	0.065	0.44	-1.71 to 3.89		
MMT	-0.31	< .01	-2.16 to -0.65		
Occlusal force	0.00	0.99	-0.01 to 0.01		

95% CI: 95% confidence interval.

MMT: Masseter muscle thickness; MMEI: Masseter muscle echo intensity.

thus also be a good indicator of dynapenia. This study also demonstrated the relationship between MMT and MMEI and showed that MMT is a useful index related to the quality and strength of the masseter muscle.

Author contributions

K. Yamaguchi and H. Tohara designed the study. K. Yamaguchi and K. Yoshimi collected the data, K. Yamaguchi analyzed the data, and K. Yamaguchi, K. Hara, A. Nakane, K. Nakagawa, S. Minakuchi, and H. Tohara interpreted the data. K. Yamaguchi drafted the manuscript, and H. Tohara and K. Hara finalized the manuscript after revision. All authors read and approved the final manuscript for submission.

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