

# Age- and sex-related differences in masseter size and its role in oral functions

Chia-Shu Lin, DDS, DPhil; Ching-Yi Wu, DDS, PhD; Shih-Yun Wu, DDS; Kai-Hsiang Chuang, DDS; Hsiao-Han Lin, MSc; Dong-Hui Cheng, DDS; Wen-Liang Lo, DDS, PhD

**T**he masseter muscle plays a key functional and structural role in the stomatognathic system. Masseter muscle activity is associated with chewing behavior<sup>1</sup> and swallowing,<sup>2</sup> and disruption in its activity may be linked to temporomandibular disorders.<sup>3</sup> The masseter is the largest jaw elevator muscle and the major contributor of the strength of jaw closure,<sup>4</sup> and its size is associated closely with bite force.<sup>5,6</sup> Cumulative evidence suggests that variation in masseter muscle size may be a critical factor related to individual differences in oral functions.<sup>4-6</sup>

Researchers have used masticatory performance (MP) and salivary flow rate (SFR) as the clinical metrics for evaluating chewing and swallowing abilities. Both a person's age and sex contribute in a critical manner to the individual differences found in MP and SFR.<sup>7,8</sup> However, researchers have not yet investigated systematically the effect of age and sex on masseter muscle size and the association of masseter muscle size with other clinical metrics. Investigators of previously published studies have reported that magnetic resonance imaging (MRI) data can be used for assessing the morphology of orofacial muscles<sup>9,10</sup> with good validity.<sup>11-13</sup> In our study, we reasoned that T1-weighted MRI data of the head, which can be acquired commonly during a brain scan, can be a suitable and convenient source for assessing masseter muscle volume (MMV).

We had 3 major goals for our study. First, we aimed to develop a voxel-based approach for assessing MMV, using T1-weighted MRI data. Second, because the investigators of previously published studies focused

## ABSTRACT

**Background.** The masseter muscle plays a key structural and functional role in the stomatognathic system. Researchers' cumulative evidence has suggested that the variation in the size of a person's masseter muscle may be a critical factor related to individual differences in oral functions. However, researchers have not yet investigated systematically the effect of a person's age and sex on masseter muscle size and the association of masseter muscle size with other clinical metrics, including masticatory performance (MP) and salivary flow rate (SFR). Using T1-weighted magnetic resonance imaging (MRI) data provides a noninvasive method for assessing masseter muscle volume (MMV).

**Methods.** Using T1-weighted MRI data, the authors developed a voxel-based method to assess MMV and investigated the associations among MMV, MP, and SFR.

**Results.** The authors acquired T1-weighted MRI data from scans of the heads of 62 healthy adults and assessed MMV by means of using a voxel-based approach. The authors' assessment results had acceptable rates of inter-rater and intrarater reliability. MMV was significantly lower in the older subgroup and in the female subgroup. In addition, the correlation for MMV was significantly positive with MP and stimulated SFR.

**Conclusions.** The study results revealed evidence that the authors' voxel-based approach, which they designed on the basis of T1-weighted MRI data, would be a reliable method for quantifying MMV.

**Practical Implications.** The findings suggest that the variation in masseter muscle size may be a critical factor to assess individual differences in oral functions.

**Key Words.** Aging; magnetic resonance imaging; mastication; masseter muscle.

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mainly on younger adults and used smaller sample sizes,<sup>14-17</sup> we determined that, by means of collecting data from a larger sample, we could investigate the age- and sex-related effects on MMV. Because previous investigators' findings revealed age-related differences in the masseter cross-sectional area<sup>18</sup> and sex-related differences in the masseter muscle thickness,<sup>19</sup> we hypothesized that a reduced MMV would be associated with older age (hypothesis 1) and female sex (hypothesis 2). Third, we investigated the association between MMV and the major clinical metrics of oral functions: MP, unstimulated SFR (uSFR), and stimulated SFR (sSFR). We hypothesized that MMV would correlate positively with MP (hypothesis 3) and that an increased MMV would be associated with increased sSFR<sup>7</sup> (hypothesis 4).

## METHODS

**Study group.** We recruited 62 healthy study participants (39 women and 23 men; age range, 23-74 years) to receive a T<sub>1</sub>-weighted MRI scan and assessments of oral functions, including MP, uSFR, and sSFR. We recruited volunteers by advertising in local community centers and the Taipei Veterans General Hospital (Taiwan). We excluded from participation volunteers who had a history of major physical or psychiatric disorders, brain injury, or brain surgery, as well as those who were unable to undergo MRI owing to physical or psychological contraindications. We received written informed consent from all participants before the experiment initiated. The institutional review boards of National Yang-Ming University (Taipei, Taiwan) and Taipei Veterans General Hospital approved the study.

**Acquisition of head T<sub>1</sub>-weighted MRI.** Using a 3 Tesla Siemens MRI scanner (Siemens Magnetom Trio, A Tim System, Siemens), we performed all of the T<sub>1</sub>-weighted MRIs at the 3T MRI Laboratory of National Yang-Ming University. We acquired the images by means of using a 32-channel head coil, using the magnetization prepared rapid gradient-echo sequence (repetition time, 2,530 milliseconds; echo time, 3.03 ms; flip angle, 7°; matrix size, 256 × 256 × 192; voxel size, 1 × 1 × 1 cubic millimeters), which we based on the protocol that we reported in our previous studies.<sup>20</sup>

**Protocol for the MMV assessment.** We assessed MMV using a voxel-based approach, which we based on the following protocol:

■ Originally, we stored the acquired images according to the Digital Imaging and Communications in Medicine standard and then transformed the images to the Neuroimaging Informatics Technology Initiative data format. We performed all investigations by means of using the imaging tool FSLView 3.2.0 (Oxford Centre for Functional MRI of the Brain).

■ One author (C.-S.L.) manually defined the anatomic region of the masseter muscle without additional between-image registration or intensity normalization.

We defined the right and left masseter muscle separately.

■ Primarily, we based the delineation of the region of interest (ROI) of the masseter muscle on the anatomic landmarks that we could clearly and repeatedly identify in a T<sub>1</sub>-weighted image (Figure 1A). First, we delineated the border of the ROI on the sagittal view from the lateral to the medial position (Figure 1A).

■ We examined the resulting masseter ROI from the axial view and the coronal view.

■ Using another software tool (fslstats, Oxford Centre for Functional MRI of the Brain), we quantified the muscle volume for each side according to the number of voxels within the defined masseter ROI. We determined the MMV value by means of calculating the average of the muscle volume from both sides.

**Reliability of the MMV assessment.** To evaluate the intrarater reliability of the method, 1 of the authors (K.-H.C.) independently assessed the MRI data of 20 study participants, by means of using the same protocol. To evaluate the intrarater test-retest reliability, 1 of the authors (C.-S.L.) performed a second assessment of the MRI data of 20 study participants 1 month after the first assessment. We quantitatively assessed both of the reliability tests, by means of using the intraclass correlation coefficient (ICC), and we qualitatively assessed the tests, by means of using the Bland-Altman plot.

**Assessment of MP.** Using the colorimetric method, we assessed MP.<sup>21</sup> After receiving the scan, each participant chewed a piece of color-changeable chewing gum (Lotte) for 3 minutes.<sup>22,23</sup> We digitized the chewed gum,<sup>24</sup> and we assessed the color change by means of using the L\*a\*b\* chromatic system.<sup>22</sup>

**Assessment of SFR.** We collected saliva by means of modifying procedures that investigators had described previously.<sup>25</sup> The study participants refrained from eating, drinking, or toothbrushing for an hour before we collected the samples. Among all the participants (N = 62), we excluded the results or did not perform an assessment in 26 participants, because those participants had eaten a meal or had a drink right before the assessment. To collect unstimulated saliva, we asked participants to drool saliva passively into a tube, the weight of which we measured, for 10 minutes after swallowing the remaining saliva. We limited study participants' movements of the head and neck regions, and we asked them to limit their speech. To obtain samples of participants' stimulated saliva, we asked the participants

**ABBREVIATION KEY.** MMV: Masseter muscle volume. MP: Masticatory performance. MRI: Magnetic resonance imaging. MW: Mann-Whitney *U* test. Q<sub>1</sub>: Lower quartile. Q<sub>3</sub>: Upper quartile. ROI: Region of interest. SFR: Salivary flow rate. sSFR: Stimulated salivary flow rate. SW: Shapiro-Wilk. uSFR: Unstimulated salivary flow rate.

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