Effect of isometric exercises on the masseter muscle in older adults with missing dentition: a randomized controlled trial

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Abstract

Maintaining oral function is important in older individuals with missing teeth for leading a healthy and independent life. This study aimed to evaluate whether simple isometric exercises can maintain and improve oral function (maximum occlusal force [MOF], masticatory ability [MA]) and masticatory muscle properties (masseter muscle thickness [MMT] and echo intensity [MMEI]) in older adults in the maintenance phase of removable prosthetic treatment.

Participants were randomly allocated into intervention and control groups. The intervention group was instructed to perform maximum clenching for 10 s, whereas the control group was instructed to tap the teeth at an arbitrary speed for 10 s. Both were repeated five times at an interval of 5 s between each activity and twice a day for 4 weeks. The outcomes were measured after a month of exercise.

The intervention group showed significant improvement in MOF, MMT during contraction, and MMEI during contraction. There was no significant difference in the MA and MMEI at rest. In the control group, no improvement was observed in any of the parameters.

When the isometric exercises were performed using a mouthpiece, there was improvement in oral function and masseter muscle properties in older individuals with Eichner B status who used dentures.

Introduction

Background and Objective

A decrease in oral function, such as maximum occlusal force (MOF) and masticatory ability (MA), has been shown to be a risk factor for adverse events, such as sarcopenia and death [1]. MOF and MA have a great influence on dietary choices [2], and a reduced bite force quotient leads to protein, fiber, mineral, and vitamin deficiency [3, 4, 5], resulting in a risk of undernutrition [6]. Maintaining oral function is important for maintaining a healthy and independent life in older individuals. Aging and tooth loss are mainly considered to be responsible for a decrease in MA and MOF [7]; however, it has been reported to be related to the properties of masticatory muscles, especially the masseter (e.g., quantity and quality). Masseter muscle thickness (MMT) is reportedly associated with MOF [8], and masseter muscle echo intensity (MMEI) is negatively correlated with MOF and MMT [9]. Muscle echo intensity can be evaluated by an ultrasonic diagnostic device that identifies non-contractile tissues, such as fat and fibrous tissue in the muscle, and indicates muscle quality [10]. Several previous studies have reported that MA, MOF, and MMT are greatly improved with dental prosthetic treatments such as implants and dentures [11–13]. In addition to prosthetic treatment, isometric exercise is used as a method of maintaining oral function. A retrospective comparative study of 28 young people showed that a simple isometric exercise using a mouthpiece resulted in improvement in MOF [14]. However, there are no reports on effective training for maintaining oral function in older adults with a missing dentition. Therefore, the purpose of this study was to clarify whether simple isometric exercises can maintain and improve oral function (MOF and MA) and masticatory muscle properties (MMT and MMEI) in older adults in the maintenance phase of removable prosthetic treatment.

Methods

Trial design

This study was a multi-center, double-blinded, randomized, controlled, parallel clinical trial. Figure 1 shows the flow diagram of this randomized, controlled trial. This study was carried out with the approval of the Clinical Research Ethics Committee of Tokyo Medical and Dental University (D2018-021) ,was registered in the UMIN clinical trial registration system on 01/08/2018 (UMIN000032933) and was conducted in accordance with the latest revision of the Declaration of Helsinki.

Participants

Participants were older adults aged 65 years and above and had removable dentures as prostheses for partial edentulism. They were recruited at an outpatient clinic (Tokyo, Japan) and at the dental hospital of Tokyo Medical and Dental University.

The inclusion criteria were as follows: Eichner B-1 to B-3 groups and patients in the maintenance phase who had already completed denture adjustment. Patients excluded from the study were those who had: 1) difficulty in following instructions, 2) progressive neuromuscular disease, 3) severe temporomandibular joint symptoms (e.g., trismus, pain during jaw movement), 4) required denture adjustment (e.g., pain with the denture), and 5) those who had teeth with mobility due to severe periodontal disease. All participants had used the current dentures for at least four weeks. Written consent was obtained from all participants after a detailed explanation of the procedure.

Intervention

At the beginning of the study, the baseline outcomes were measured, and the impression of the upper jaw for the mouthpiece was made. A mouthpiece was fabricated by softening a 1.0-mm-thick thermoplastic sheet (for soft splint), which was then adapted on a plaster model with the help of a vacuum adapter, as described in a previous study [14]. On the next visit, the participants received the mouthpieces after proper adjustment and were randomly divided into two groups. After the allocation, one of the dentists explained the intervention method, and another measured the baseline outcomes.

Intervention group

In the intervention group, a denture was provided to replace missing teeth in either the upper or lower jaw, and then a mouthpiece was fabricated for the upper jaw. If there was a denture in the upper jaw, the mouthpiece was worn over the denture. Subsequently, the participant was instructed to perform maximum clenching for 10 s. The training was repeated five times at an interval of 5 s between each clenching activity.

Control group

In the control group, a denture was provided to replace missing teeth in either the upper or lower jaw, and then a mouthpiece was fabricated for the upper jaw. If there was a denture in the upper jaw, the mouthpiece was worn over the denture. However, unlike the intervention group, the participant was instructed to tap his/her teeth at an arbitrary speed for 10 s, and this training was repeated five times at an interval of 5 s.

Both the groups performed their respective exercises twice a day, in the morning and evening, for a period of 1 month.

Measurement of MOF, MA, MMT, and MMEI

MOF, MA, MMT, and MMEI were measured using appropriate diagnostic devices. Participants were instructed to be seated on chairs in a relaxed position with their backs stretched, feet firmly touching the floor, and the Frankfurt horizontal plane parallel to the floor.

To measure the MOF, a pressure-sensitive film (Dental Prescale II, GC, Tokyo, Japan) was aligned with the dentition, and the participant was instructed to bite with maximum force. The scanner was calibrated [15], the pressure-sensitive film was fitted to the template, and analysis was performed with analysis software (Bite Force Analyzer GC, Tokyo, Japan).

For measurement of MA, participants were instructed to freely chew a cylindrical-shaped gummy jelly (GC, Tokyo, Japan) composed of 40% maltose, 10% sorbitol, and 5% glucose, for 20 s. After chewing, the participants were asked to hold 10 mL of distilled water in their mouth and to spit the gummy jelly, distilled water, and saliva into a cup with a filter. The glucose concentration in the filtrate was measured using a glucose measuring device (Glucosensor GS-II, GC, Tokyo, Japan) [16].

An ultrasonic diagnostic device (fST9600 Lequio Power Technology, Naha, Japan) was used to measure MMT and MMEI. A linear probe was used with a broadband frequency ranging from 6.7 MHz to 8.0 MHz. Scanning was performed in the B mode. The scanning depth was 38 mm and the gain was 80 dB, and these values were constant during the measurement.

As described in a previous study [17], the probe was placed parallel to the mandibular margin, approximately midway between the zygomatic arch and the mandibular angle, and perpendicular to the skin surface. While measuring the thickness of the masseter muscle and its echo intensity, the patients were instructed to maintain the mandibular rest position for at least 20 min before ultrasonography in order to reduce the effects on blood flow and interstitial fluid due to muscle contraction [18].

Ultrasound images were analyzed using Image-J software version 1.49 (National Institutes of Health, Bethesda, MD), and the maximum distance from the exterior portion of the ramus to the masseteric fascia was measured as MMT (Fig. 2). When measuring the MMEI, the region of interest was set to include the entire masseter muscle (Fig. 2). Echo intensity is attenuated due to the influence of subcutaneous fat; therefore, the following correction calculation was performed:

Corrected echo intensity = uncorrected echo intensity + subcutaneous fat thickness [cm] × 40.5278

The corrected masseter muscle echo intensity was used as the measured value [10, 19]. Subcutaneous fat thickness was measured at three locations and the average of the three measurements was used for calculations.

Sample size

G * Power software version 3.1 (Kiel University) was used to calculate the sample size. The effect size was set to 0.8, with reference to previous studies [11, 14] (d = 0.65, 0.88) [20]. When the α value was set to 0.05, detection power was set to 0.8, and effect size d to 0.8, the required number of samples was 52. The number of dropouts was expected to be 8% for a total of 56 people.

Randomization

The sampling followed a randomized stratified block method that combined a stratified method (gender) and a block method (intervention, control). One dentist was in charge of the outcome measurement, while the participants who received the dentures and mouthpieces were trained by another dentist. In other words, the dentists who measured the outcome and the dentist who explained the allocation and intervention methods were different. Therefore, both the research participants and the dentists who measured the outcomes were blinded.

Statistical method

The Shapiro-Wilk test was performed on each measured value to confirm its normality. The Mann-Whitney U-test was performed for each baseline measurement item. The Wilcoxon signed rank test was used for pre- and post-intervention comparisons of study participants in each group, and the Mann-Whitney U-test was used for comparison between the two groups.

Results

Participants

Research participants were recruited from January 2019 to September 2020. Fifty-six participants were randomly assigned to two groups. Three dropped out of the study because of anxiety about hospital visits during the ongoing coronavirus pandemic, and the final number of participants was 53 (Fig. 1). Table 1 shows the participant backgrounds for both groups.

Table 1
Baseline characteristics of the participants (n = 56)

	Group		Total	*P* value
	Control(n = 28)	Intervention(n = 28)		
Age(years)	74.86(5.942)	72.96(6.608)	73.91(6.299)	0.265
Gender				
Male	16	16		
Female	12	12		
Body height	156.32	158.64	157.48	0.218
Body weight	53.98	58.34	56.16	0.086
BMI	22.029	23.164	22.596	0.165
Eichner				
B1	6	7		
B2	6	11		
B3	16	10		
MA (mg/dL)	181.19(54.872)	175.54(62.81)		0.799
MOF(N)	596.37(310.31)	727.84(366.29)		0.432
rMMT(mm)	0.76(0.18)	0.90(0.22)		0.030*
cMMT (mm)	1.02(0.26)	1.17(0.26)		0.213
rMMEI	158.87(19.42)	145.45(34.15)		0.077
cMMEI	136.41(22.71)	128.63(31.18)		0.481
*p-value < 0.05				
MA: masticatory ability				
MOF: maximum occlusal force				
rMMT: masseter muscle thickness at rest				
cMMT: masseter muscle thickness during contraction				
rMMEI: masseter muscle echo intensity at rest				
cMMEI: masseter muscle echo intensity during contraction				

Outcome

At the baseline measurement stage, no significant difference was observed except in MMT at rest (rMMT) (Table 1). After the intervention, as compared to the control group, the intervention group showed significant

improvement in MOF (P = 0.001), MMT during contraction (cMMT) (P = 0.022), and MMEI during contraction (cMMEI) (P = 0.017). There was no significant difference in MA and MMEI at rest (rMMEI) (Table 2).

Table 2
Comparison between control (n = 27) and the intervention groups (n = 26) before and after intervention

	Baseline		**At 4weeks' post intervention**		***p* value**
	Control(n = 28)	Intervention(n = 28)	Control(n = 27)	Intervention(n = 26)	
MA (mg/dL)	181.19(54.872)	175.54(62.81)	185.19(60.92)	197.92(52.89)	0.557
MOF(N)	596.37(310.31)	727.84(366.29)	609.49(346.17)	962.70(420.29)	0.001*
rMMT(mm)	0.76(0.18)	0.90(0.22)	0.74(0.19)	0.92(0.21)	0.007*
cMMT (mm)	1.02(0.26)	1.17(0.26)	1.04(0.29)	1.23(0.27)	0.022*
rMMEI	158.87(19.42)	145.45(34.15)	151.70(34.80)	138.61(42.81)	0.085
cMMEI	136.41(22.71)	128.63(31.18)	130.75(32.03)	115.82(36.17)	0.017*
*p-value < 0.05					
MA: masticatory ability					
MOF: maximum occlusal force					
rMMT: masseter muscle thickness at rest					
cMMT: masseter muscle thickness during contraction					
rMMEI: masseter muscle echo intensity at rest					
cMMEI: masseter muscle echo intensity during contraction					

Significant improvement in MOF (P < 0.001), cMMT (P = 0.003), cMMEI (P = 0.006), and MA (P = 0.016) was observed in the intervention group on comparison following intervention. In the control group, no improvement was observed in any of the measured values (Table 3).

Table 3
Within-group comparison before and after intervention

	control		*p* value	intervention		*p* value
	baseline	post intervention		baseline	post intervention	
MA (mg/dL)	181.19(54.872)	185.19(60.92)	1	175.54(62.81)	197.92(52.89)	0.016*
MOF(N)	596.37(310.31)	609.49(346.17)	0.683	727.84(366.29)	962.70(420.29)	0.000*
rMMT(mm)	0.76(0.18)	0.74(0.19)	0.242	0.90(0.22)	0.92(0.21)	0.283
cMMT (mm)	1.02(0.26)	1.04(0.29)	0.99	1.17(0.26)	1.23(0.27)	0.003*
rMMEI	158.87(19.42)	151.70(34.80)	0.079	145.45(34.15)	138.61(42.81)	0.501
cMMEI	136.41(22.71)	130.75(32.03)	0.471	128.63(31.18)	115.82(36.17)	0.006*
*p-value < 0.05						
MA: masticatory ability						
MOF: maximum occlusal force						
rMMT: masseter muscle thickness at rest						
cMMT: masseter muscle thickness during contraction						
rMMEI: masseter muscle echo intensity at rest						
cMMEI: masseter muscle echo intensity during contraction						

Discussion

In this intervention study, the following two points were observed. On performing isometric exercises using a mouthpiece, there was (1) improvement in oral function and (2) improvement in masseter muscle properties in older individuals with Eichner B status who used dentures.

First, as a result of training, the MOF was greatly improved. There are many reports of resistance training for limb muscles, which increases muscle mass and strength [21, 22]. In general, with advancing age, there is muscular atrophy and decline in muscle mass, strength, and physical function, which has been termed sarcopenia [23]. On the other hand, it has been reported that significant improvement in MOF is achieved by resistance training in adults without missing teeth [14]. The masseter tissue is dominated by Type 1 muscle fibers and has a different composition from the quadriceps femoris, which has a large number of Type 2 muscles [24]. Type 1 muscle fibers are more susceptible to disuse than aging [25]. Tooth loss is also an independent factor related to MMT [26]. Furthermore, it has been reported that chewing exercises for older people with 24 or more remaining teeth improved MOF [27]. In this study, we targeted older adults with missing teeth and found an improvement in MOF, which was consistent with previous studies [14, 27].

The pre- and post-intervention MA was significantly different in the intervention group, but not in the control group. As the occlusal support of the molars decreases, MA is greatly affected by the salivary flow [28] and tongue [29, 30]. The lack of significant difference in MA could be probably due to the target muscle of the training being the masseter, and not the tongue.

Second, an improvement in the properties of the masseter muscle was observed. Significant improvements were observed in both cMMT and cMMEI. It has been found that age-related changes strongly affect muscle strength and muscle mass [31]. Muscle weakness is a risk factor for subsequent hospitalization and death [32]. Many reports have shown that resistance training is useful for improving muscle strength, and muscle mass is gained after strength improvement [33]. Early muscle hypertrophy is generally considered to take at least 6 weeks [34]. However, in this study, a significant improvement in functional muscle hypertrophy was observed at 4 weeks. One of the factors that causes muscle hypertrophy at a relatively early stage is the high sensitivity of the masseter muscle to testosterone. Testosterone has the effect of promoting protein synthesis in muscle [35]. In a study of rats, injection of testosterone increased masseter muscle mass by 38%, which was reportedly more sensitive than other muscles [36]. Isometric exercise has been shown to significantly increase testosterone levels [37, 38]. The masseter may be a muscle that is more affected by isometric exercise compared to other muscles.

In this study, the cMMEI was also significantly improved. The higher the number, the more non-contractile tissue in the muscle, indicating a decrease in muscle quality. Previous studies in healthy older adults have shown a strong negative correlation between cMMT and cMMEI [9]. It is considered that the improvement of cMMT by isometric exercise also led to an improvement in cMMEI.

This study has several limitations. First, this study targeted older people in the Eichner B group, with limited number of missing teeth. In order to clarify the usefulness of isometric exercises for the masseter muscle, it will be necessary to conduct studies on patients with multiple missing teeth, including the Eichner C group. Second, the training period for this study was 1 month, and long-term effects were not considered. Tracking the long-term effects of isometric exercises is warranted in future research.

Significance And Future Implications

In this study, isometric exercise using a mouthpiece was shown to significantly improve occlusal force and masseter muscle properties (quantity and quality) in older people using Eichner B dentures. Masseter muscle hypertrophy was confirmed in a short span of 1 month, indicating that the masseter muscle is more responsive to isometric exercise than other muscles. Therefore, it is possible to sufficiently improve and maintain oral function even for older individuals who are in the maintenance phase after the completion of prosthetic treatment. The possibility of self-management of oral function and masticatory muscle properties has been demonstrated in this study.

Declarations

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Authors' contributions

S.T., K.Y. and H.T. designed the study. S.T. collected and analyzed the data. S.T., K.Y., K. Y., A. N., K.N., T.O., and H.T. interpreted the data. S.T. drafted the manuscript, and K.Y. and H.T. finalized the manuscript after revision. All authors have read and approved the final manuscript for submission.

Competing interests

The authors state that there are no conflicts of interest related to this article.

References

(1) Inomata, C., *et al*. Significance of occlusal force for dietary fibre and vitamin intakes in 4 independently living 70-year-old Japanese: from SONIC study. *J. Dent*. **42**,556-564 (2014).

(2) Nowjack-Raymer, R. E. & Sheiham, A. Association of edentulism and diet and nutrition in US adults. *J. Dent. Res.* **82**, 123-126 (2003).

(3) Borie, E., *et al*. Maximum bite force in elderly indigenous and non-indigenous denture wearers. *Acta Odontol. Latinoam.* **27**, 115-119 (2014).

(4) Moynihan, P., *et al*. Researching the impact of oral health on diet and nutritional status: methodological issues. *J. Dent.* **37**, 237-249 (2009).

(5) Nowjack-Raymer, R. E. &Sheiham, A. Numbers of natural teeth, diet, and nutritional status in US adults. *J. Dent. Res*. **86**, 1171-1175 (2007).

(6) Houston, D. K., *et al*. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the health, aging, and body composition (Health ABC) study. *Am. J. Clin. Nutr.* **87**1,50e5 (2008).

(7) Okamoto, N., Amano, N., Nakamura, T. & Yanagi, M. Relationship between tooth loss, low masticatory ability, and nutritional indices in the elderly: a cross-sectional study. *B.M.C. Oral Health***. 19**, 110 (2019).

(8) Raadsheer, M. C., van Eijden, T. M., van Ginkel, F.C. & Prahl-Andersen, B. Contribution of jaw muscle size and craniofacial morphology to human bite force magnitude. *J. Dent. Res.* **78,** 31-42 (1999).

(9) Yamaguchi, K., *et al*. Factors associated with masseter muscle quality assessed from ultrasonography in community-dwelling elderly individuals: A cross-sectional study. *Arch. Gerontol. Geriatr.* **82**, 128-132 (2019).

(10) Young, H. J., Jenkins, N. T., Zhao, Q. & Mccully, K. K. Measurement of intramuscular fat by muscle echo intensity. *Muscle & Nerve*. **52***)*, 963-971 (2015).

(11) Melo, A. C. M., Ledra, I. M., Vieira, R. A., Coró, E. R. & Sartori, I. A. M. Maximum bite force of edentulous patients before and after dental implant rehabilitation: Long-term follow-up and facial type Influence. *J. Prosthodont*. **27**, 523-527 (2018).

(12) Müller, F., Duvernay, E., Loup, A, Vazquez, L., Herrmann, F. R. & Schimmel, M. Implant-supported mandibular overdentures in very old adults: a randomized controlled trial. *J. Dent. Res*. **92**, 154S-160S (2013).

(13) von der Gracht, I., Derks, A., Haselhuhn, K. & Wolfart, S. EMG correlations of edentulous patients with implant overdentures and fixed dental prostheses compared to conventional complete dentures and dentates: a systematic review and meta-analysis. *Clin. Oral Implants Res*. **28**, 765-773 (2017).

(14) Thompson, D. J., Throckmorton, G. S. & Buschang, P. H. The effects of isometric exercise on maximum voluntary bite forces and jaw muscle strength and endurance. *J. Oral Rehab*. **28** 909-917 (2001).

(15) Shiga, H., *et al.* Comparison of two dental pre scale systems used for the measurement of occlusal force. *Odontology*. **108**, 676-680 (2020).

(16) Takeshima, T., Fujita, Y. & Maki, K. Factors associated with masticatory performance and swallowing threshold according to dental formula development. *Arch. Oral Biol.* **99**, 51-57 (2019).

(17) Serra, M. D., Duarte Gavião, M. B., & dos Santos Uchôa, M. N. The use of ultrasound in the investigation of the muscles of mastication. *Ultrasound Med. Biol*. **34**, 1875-1884. (2008).

(18) Yoshiko, A., Kaji, T., Sugiyama, H., Koike, T., Oshida, Y. & Akima, H. Effect of 12-month resistance and endurance training on quality, quantity, and function of skeletal muscle in older adults requiring long-term care. *Exp. Gerontol.* **98**, 230-237 (2017).

(19) Stock, M. S., Whitson, M., Burton, A. M., Dawson, N. T., Sobolewski, E. J. & Thompson, B. J. Echo Intensity Versus Muscle Function Correlations in Older Adults are Influenced by Subcutaneous Fat Thickness. *Ultrasound Med. Biol.* **44**, 1597-1605 (2018).

(20) Cohen, J. A Power Primer. Psycohol. Bull. **112**, 155-159 (1992).

(21) Wang, E., *et al*. Impact of maximal strength training on work efficiency and muscle fiber type in the elderly: Implications for physical function and fall prevention. *Exp. Gerontol*. **91**, 64-71 (2017).

(22) Abe, T., DeHoyos, D. V., Pollock, M. L. & Garzarella, L. Time course for strength and muscle thickness changes following upper and lower body resistance training in men and women. *Eur. J. Appl. Physiol.* **81**, 174-180 (2000).

(23) Cruz-Jentoft, A. J., *et al.* Sarcopenia: revised European consensus on definition and diagnosis. Writing group for the European working group on sarcopenia in Older People 2 (EWGSOP2), and the Extended Group for EWGSOP2. *Age Ageing*. **48**, 16-31 (2019).

(24) Monemi M., Eriksson P. O., Eriksson, A. & Thornell, L. E. Adverse changes in fibre type composition of the human masseter versus biceps brachii muscle during aging. J*. Neurol.Sci.* **154**, 35-48 (1998).

(25) Cullins, M. J. & Connor, N. P. Alterations of intrinsic tongue muscle properties with aging. *Muscle Nerve*. **56**, E119-E125 (2017).

(26) Yamaguchi, K., *et al.* Relationship of aging, skeletal muscle mass, and tooth loss with masseter muscle thickness. *B.M.C. Geriatr.* **18**, 67 (2018).

(27) Kim, M. J., *et al.* Effects of chewing exercises on the occlusal force and masseter muscle thickness in community-dwelling Koreans aged 65 years and older: a randomized assessor-blind trial. *J. Oral Rehabil.* **47**, 1103-1109 (2020).

(28) Ikebe, K., et al. Masticatory performance in older subjects with varying degrees of tooth loss. *J. Dent*. **40**, 71-6 (2012).

(29) Koshino, H., Hirai, T., Ishijima T. & Ikeda, Y. Tongue motor skills and masticatory performance in adult dentates, elderly dentates, and complete denture wearers. *J. Prosthet. Dent.* **77**, 147-152 (1997).

(30) Sagawa, K., *et al*. Tongue function is important for masticatory performance in the healthy elderly: a cross-sectional survey of community-dwelling elderly. *J. Prosthodont. Res* **63**, 31-34 (2019)

(31) Goodpaster, B. H., *et al.* The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. J*. Gerontol. A. Biol. Sci. Med. Sci.* **61**, 1059-1064 (2006).

(32) Fried, L. P., et al. Cardiovascular Health Study Collaborative Research Group. Frailty in older adults: evidence for a phenotype. *J. Gerontol. A. Biol. Sci. Med. Sci.* **56,** M146-56 (2001).

(33) de Mello, R. G. B., Dalla Corte, R. R., Gioscia, J. & Moriguchi, E. H. Effects of physical exercise programs on sarcopenia management, dynapenia, and physical performance in the elderly: a systematic review of randomized clinical trials. *J. Aging. Res*. **2019**,1959486 (2019).

(34) Staron, R. S., et al. Skeletal muscle adaptations during early phase of heavy-resistance training in men and women. *J. Appl. Physiol.* **76,** 1247-1255 (1994).

(35) Griggs, R. C., Kingston, W., Jozefowicz, R. F., Herr, B. E., Forbes, G. & Halliday, D. Effect of testosterone on muscle mass and muscle protein synthesis. *J. Appl. Physiol*. **66,** 498-503 (1989).

(36) Widmer, C. G. & Morris-Wiman, J. Limb, respiratory, and masticatory muscle compartmentalization: developmental and hormonal considerations. *Prog. Brain Res*. **187**, 63-80 (2010).

(37) Kraemer, W. J. & Ratamess, N. A. Hormonal responses and adaptations to resistance exercise and training. *Sports Med.* **35**, 339-361 (2005).

(38) Vingren, J. L., Kraemer, W. J., Ratamess, N. A., Anderson, J. M., Volek, J. S. & Maresh, C. M. Testosterone physiology in resistance exercise and training: the up-stream regulatory elements. *Sports Med.* **40**, 1037-1053 (2010).

Figures

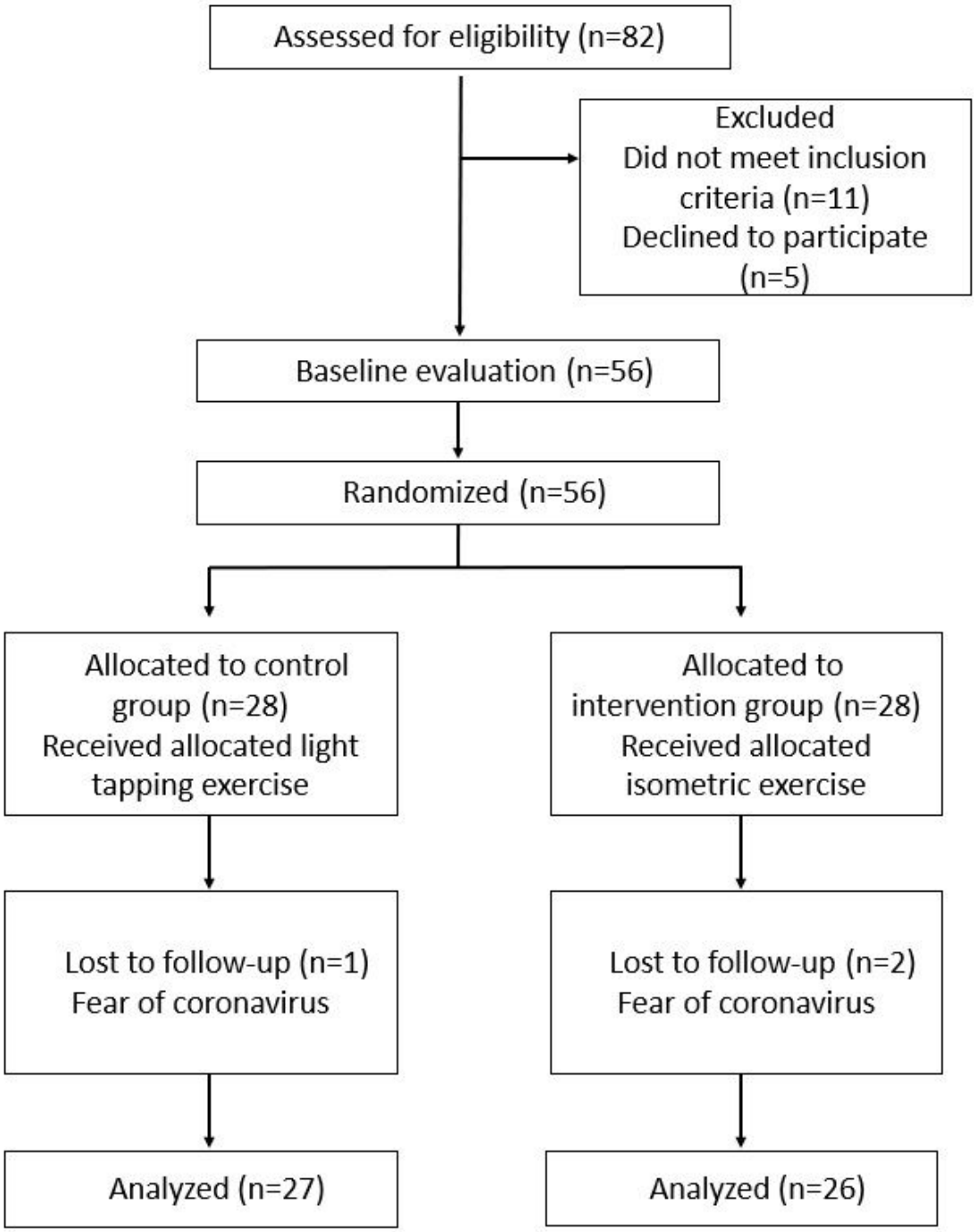

Figure 1

Flow diagram of this randomized-controlled trial.

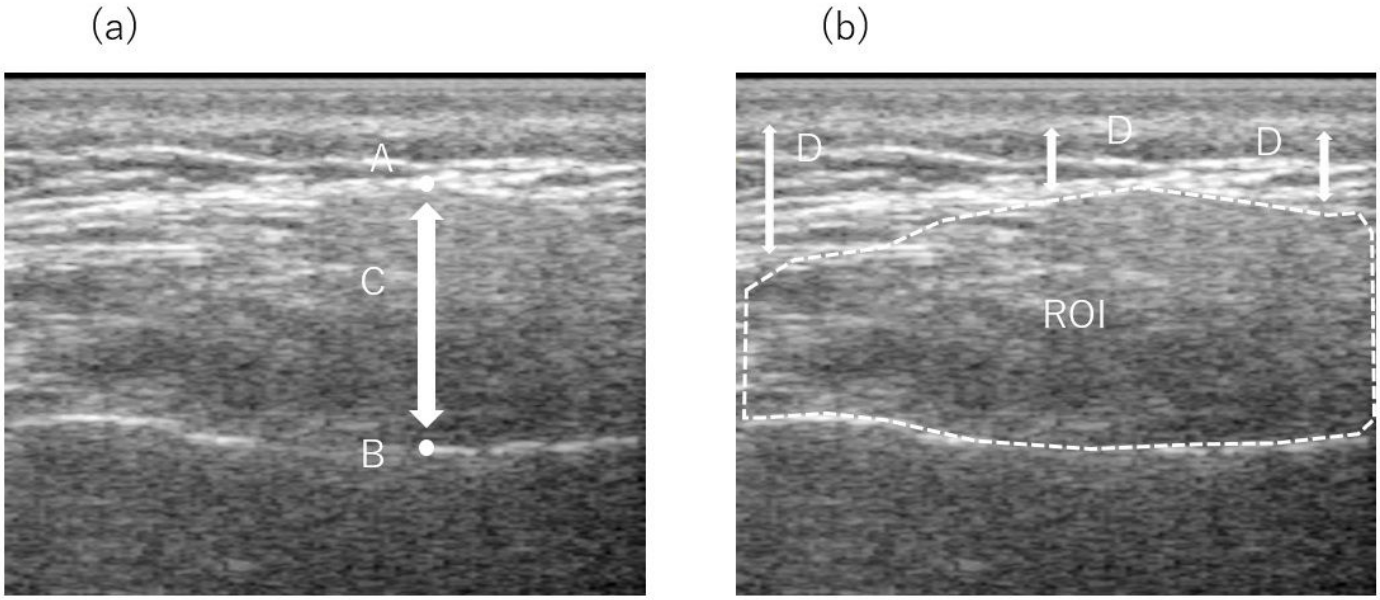

Figure 2

Masseter muscle evaluation by ultrasound diagnostic apparatus. (a) Masseter muscle thickness（MMT） measurement. (b) Masseter muscle echo intensity（MMEI）measurement. (A) Masseter muscle surface (B) Mandibular ramus (C) Masseter muscle thickness (D) subcutaneous fat thickness. ROI: region of interest