Correlation between ultrasound normal values of the masticatory muscle masseter and maximal bite force in a healthy adult population

Fontys University of Applied Science

Eindhoven, The Netherlands

Bachelor Thesis
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Preface:

This study took place in Fontys, University of Applied Sciences, Eindhoven. The project began in September 2014 and ended in January 2015 for a total of 20 weeks fully dedicated to designing the research, carrying out the experiment and writing the conclusion.

In the light of this, I would like to express my gratitude to my supervisor Marc Schmitz for his advice, availability and guidance through this challenging and rewarding journey. Marc was able to convey to us his enthusiasm for the study and the technology involved, generating and driving motivation amongst all the researchers. I also want to thank the research group I worked with. Getting to work with the speech therapy team and my PT colleagues has been nothing but a great experience. In particular I want to thank Virpi Rajala, for being my ultrasound partner and spending with me several hours of work on the assessment of the pictures taken for the purpose of this study. She never missed the chance to encourage me in pursuing our final goal.

I would also like to thank my second assessor, Patrick Theeven for the help and the significant "statistical" advice he has given to me for the improvement of my research, and Marja Vermust for her great organization skills and for her ability to solve the student's issue, including mine.

Last but not least I would like to thank all the members of my family who continuously challenged me to improve and who always supported me giving me that confidence I did not know I had.

In thanking my family I am also thanking my closest friends who deserve my whole gratitude. In such a challenging time, where I have not always had the best mood and attitude they have always been there day and night showing their empathy and support. I am lucky enough to say that I never missed a sincere hug.

Abstract

Poor mastication has been widely associated with cognitive impairments in elderly people. Ultrasound technology (US) and bite force measuring devices are both used to assess the masticatory muscles, providing an indication of their condition.

Objective: The purpose of this study was to assess masseter muscle transversal thickness and the magnitude of maximal bite force (MBF) in a group of healthy young adults.

Design: Participants in the study consisted of 126 individuals (67 females and 59 males; aged 25.1±4.8 years) without any jaw disorder. MBF was determined with a transducer placed in between incisors and the transversal thickness of the masseter muscle was evaluated bilaterally for both relaxed and contracted state of the masseter muscle by using ultrasound (US) technology. The contracted state of the muscle was achieved by asking the participants to forcefully clench on their molars. The normality of distribution of all the data was assessed by Shapiro-wilk test. A comparison of the mean of three MBF measurements and three US measurements of the contracted masseter muscle was made using the Pearson correlation analysis. Since the population was heterogeneous, inter-gender difference was determined for both methods.

Results: There was no difference between left and right masseter muscle thickness, but significant was the difference between contracted and relaxed state of the muscle. Furthermore, significant difference was observed for both MBF and US outcome measures between men and women. Muscle thickness was poorly correlated to MBF r= .3, $P < .05$. When the relation between US and MBF measurements was analyzed with intra-gender discrimination the analysis would still show a very weak correlation.

Table of Contents

1 Introduction

Across the globe, life expectancy has increased dramatically over the past century. However, this salutary trend also has a negative aspect, as the common chronic diseases of older people are becoming more prevalent. (1) The number of people affected by Alzheimer's disease (AD) was 26.6 million worldwide in 2006. Normal aging is associated with impairments of cognitive functions, such as memory and attention (2) and the prevalence of dementia increases due to the global aging of the population. (1)

Among all the risk factors to develop dementia, tooth loss is surprisingly one of them. (3) Several previous studies found out that loss of teeth is associated with poor mastication, malnutrition and cognitive impairments. (2–4)

Recent studies in functional magnetic resonance imaging (fMRI) and positron emission tomography (or PET) revealed that mastication increases cortical blood flow and widely activates various cortical areas of the somatosensory, supplementary motor, and insular cortices, as well as the striatum, thalamus and cerebellum. (5) These brain areas are responsible of cognitive skills such as memory and attention.

Mastication is a very complex biomechanical process where the masseter muscle (or m. masseter) is one of the four principal muscles of mastication. (6) The function of masseter is to elevate the mandible, thus it closes the mouth, and to approximate the teeth. Mastication has been shown to promote and maintain general health and cognitive functions, thus preserving masticatory function is essential for good quality of life. (7,8)

Different techniques have been used to clinically assess the physiological and functional characteristics of the masticatory muscles. One way is the measurement of maximal bite force (or MBF). (8) MBF is a useful and direct indicator of the functional state of the masticatory muscles and the force results from the action of the jaw elevator muscles (firstly m. masseter). (9) The assessment of the bite force, and its recordings, can be made directly by using a suitable transducer placed in between a pair of teeth. (10)

An alternative method is indirect evaluation of the bite force by engaging other physiological variables known to be functionally related to the force production. (11,12) Thickness of the muscle represents one of these physiological variables, and it can be assessed by means of ultrasound technology. (8,12–17) Ultrasound (or US) has been shown to be very effective in morphological analysis of soft tissue and in measuring thickness of human muscles in vivo as indication of muscle size. (13)

Previous studies have shown that US is an important tool for evaluating the functional capacity of m. masseter contraction during dental clenching. (8,12,13,15,16) This states US as a tool, which does not only observe the morphology of the muscle but also its structural changes during contraction. (12,13,18)

It is clear that MBF indicates the functional state of the masticatory muscles, by only assessing the force exerted by these specific muscles. (10–13,19)

In praxis the evaluation of bite strength is done by using a specific device which reports the force of the bite in kilograms. (10,11,19) In the Netherlands the device meant for the evaluation of the bite force was introduced into the market in 2010 and since then only four devices were sold to four different institutes[1] making the MBF device not easily available to the general population as a consequence. The MBF measurement tool might also be difficult to use on elderly patients because of the invasive and effort-related nature of the test

[1] *Data obtained by calling the manufacturer of the bite force device (VU-BFG) used for this research*.

procedure. (11,19) During the assessment of MBF, the patient is in fact asked to bite as strong as he can on the probe causing discomfort or even pain, which might alter the reliability of the test results. (10–12,19,20)

US, instead, enables not only a morphological evaluation of the muscle tissue, but also a functional one. It is a dynamic technique and, therefore, capable of visualizing normal and pathological muscle movements. (17)
US is a non-invasive (no cumulative biological effects) and real-time technique capable of scanning the muscles while performing functional movements and it gives the possibility to quickly evaluate differences of the same muscle or muscle group between left and right side of the body. (21)
Additionally, it has several advantages over similar imaging techniques (magnetic resonance imaging and computerized tomography) because it is rapid, inexpensive, the equipment can be easily handled and transported. (12-14)
The use of US to examine m. masseter strength may permit a better understanding of the participation of this specific muscle to the mastication, serving as guideline for the diagnoses and possible therapeutic approach that could be offered to patients with masticatory muscle deficit. (12)
Many are the studies comparing m. masseter, and the other masticatory muscles, to bite force in a population with complaints and/or elderly and how anthropometrics such as gender, age, body mass index and craniofacial dimensions influence both outcome measures. (11,12,20,22–24)
However, the relationship between these two types of “masticatory assessments” has been poorly investigated in healthy adult subjects and in a time-spam where bite strength is fairly constant (aged 20 to 40). (9,10)
In the light of this, the purpose of this study is to assess human mastication by using two measuring tools, a bite force gauge and US technology; one will assess the force human bite exerts and the other one will look at the muscle masseter morphology during contraction and therefore observe how related are their outcomes. What is the correlation between m. masseter transversal thickness and the magnitude of bite force in an adult, complain-free population?

1.1 Research question

What is the correlation between thickness of the masseter muscle and maximal bite force?

2 Method/ study design

This is a cross-sectional study, where two different types of measuring tools, the ultrasound measuring muscle thickness in millimetres (mm.) and the bite force gauge measuring strength in kilograms (kg) are used to assess m. masseter, in order to determine their correlation.

2.1 Participants recruitment

126 medically healthy young adults (59 males and 67 females) participated to the study.

Participants were selected by means of inclusion and exclusion criteria. As inclusion criteria participants had to be healthy young adults aged between 20-40 years old, with no history of temporo-mandibular joint problems and capable of speaking and understand the English language. The exclusion criteria were any history of jaw and chewing problems in the past 6 months, jaw or jaw muscles operation recorded in subject's medical history, individuals who cannot undergo measurement tests, individuals with those cognitive problems that hinder awareness of his/her participation.

The experiment concerned two different types of measuring tools: the ultrasound technology measuring muscle thickness in mm., and the bite force gauge measuring strength in kg are used to assess m. masseter, in order to determine their correlation.

One investigator will assess muscle masseter thickness in a relaxed state and in a contracted one. Therefore the outcome measure will be the thickness in mm. in both stands.

One other investigator will then assess maximal bite force. The outcome measure will then be m. masseter strength in kg.

2.2 Ethical Paragraph

The participants were informed about the testing procedure via invitation letter (Appendix I) where they were also informed about the handling of personal data. Additionally, they were asked to sign an informed consent form (Appendix II) before they will be assessed.

The "Fontys Ethics Committee" approved this study. The data of the participants have been coded and stored in Excel and SPSS files. Access is solely granted to Maaske Treurniet (speech therapists supervisor), Marc Schmitz (physiotherapy supervisor) and Jaap Jansen (head of research: musculoskeletal research physical therapy). The data of the research will be anonymously saved for 15 years and the encryption key will always be saved within Fontys, Paramedical School of Eindhoven.

2.3 Measuring tool I

VU Bite Force Gauge (or VU BFG)

The maximal bite force (MBF) is measured with the VU University Bite Force Gauge (VU-BFG). The VU-BFG is a hand-held device, which uses a load cell to measure maximal voluntary bite force in kilograms (Developer: Dekker, E. (2010). Serial number: 2010003).

Participants were instructed to bite as long and hard as possible and were encouraged continuously during the sampling. (22)

The investigator (speech therapy student) assessed MBF of 126 individuals who voluntarily participated to the experiment. MBF data were collected with the subjects sitting in an upright position, with the head in a natural posture. All the participants received a brief instruction about the procedure of the testing. The measuring tool used for this experiment had a probe of 6 mm. covered by thick black rubber pad of 2x2 mm. to protect the teeth from any potential damage. In order to guarantee hygiene, a blue plastic sleeve was applied to the probe and changed each time a new participant was examined. (Figure 1)
The investigator disinfected her hands before executing the measurements.

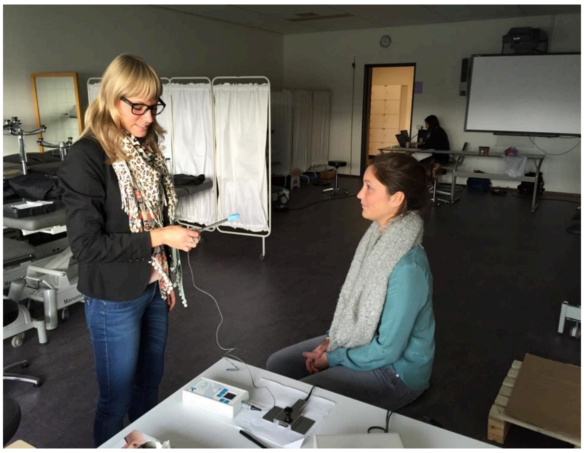

Figure 1. Investigator gives a brief instruction before starting with the testing. The investigator can keep track beginning and ending of the participant's performance by looking at the device, which informs when the participant can stop biting and it assess the magnitude of the bite in about 20 s and shows it in quick time display.

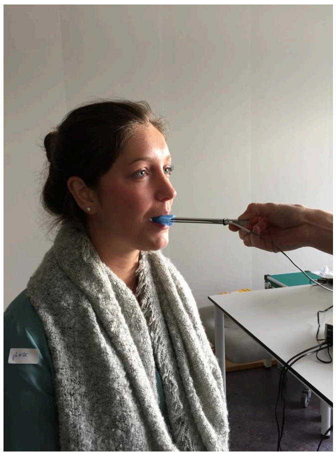

Figure 2. BFG probe held by the investigator and placed in between incisors for bite force measurements

The experiment began with the investigator placing the probe into each participant ' s mouth, in between incisors as showed in Figure 2. The participants were then asked to bite on the probe as forcefully as possible for about 2 s and verbal encouragement was used to support the volunteers' performance. Three repetitions were completed with a 20 s rest interval between repetitions. Each participant took about 10 minutes for the whole testing procedure.
The procedure for the use of the UV BFG is accurately described in the protocol (Appendix III).

2.3 Measurement tool II

Ultrasound (US) Technology: MyLab^{TM}One by Esaote .

The evaluation of the masseter muscle thickness was conducted using the MyLab^{TM}One system (developer: ESAOTE 2010, Serial-number 112810000), provided of a 13MHz linear transducer, which was positioned on the skin surface over approximately one third (from the genial angle) of the m. masseter, positioned just perpendicular to the direction of the muscle fibres (Figure 3a./ 3b.)

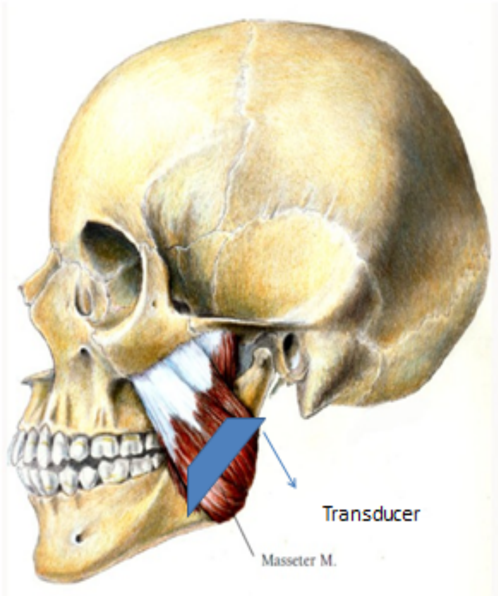

Figure 3a. Transducer on the lower 1/3 of masseter muscle

Figure 3b. Transducer position during testing

Agreement was made among researchers that to have a proper transversal view of the muscle thickness, the transducer would be positioned as it follows: on the ear level, with its sided light pointing at the ear lobe, and the transducer body just parallel to the jaw line. Basically, it was placed at the lower one-third between zygomatic arch and mandibular. Even though an agreement was made, the positioning of the transducer could never be totally standardized. Individuals different parameters, f.e. different cranio-facial dimension would have had a bad influence on a standardized transducer position. Therefore agreement on the anatomical landscape was still kept, but only confirmed by the visual feedback of the image displayed in the US screen.
Airtight inert gel was used to guarantee proper visualization of the US images. Light pressure was applied when positioning the transducer on the participants' skin; too much pressure would have caused the muscle to be squeezed leading to confounded outcomes.

One investigator performed the assessment of 126 participants. Each participant started the test either with the US investigation or with the MBF one. The ones initiating the test we marked with an asterisk next to their assigned number to distinguish them from the ones starting with MBF.
The US investigator started the procedure by introducing herself to each participant and by giving them a quick, but clear explanation of the US machine, assuring them that it is a safe and harmless diagnostic tool with a non–invasive nature (no radiation) and real-time display (11,12,20,23) that returns the thickness of the muscles in millimetres.

The testing procedure had the investigator to register 12 images of the transversal thickness of the masseter muscle (Figure 4).

Six measurements were taken for each side of the face; three images of the m. masseter in a relaxed state and another three images of the same muscle in a contracted state. To obtain a relaxed m. masseter, participants were instructed to maintain a slight interocclusal position, lip contact and to avoid moving during the testing. To obtain a contracted m. masseter the participants were told to gradually and forcefully clench (maximal intercuspal position) and to steadily maintain this position until image was taken.

Figure 4. Each participant masseter muscle was assessed for both left and right side and in both relaxed and contracted state or a total of 12 images per participant.

US machine had standard settings: gain 70/E0/100%, 13 MHz, and depth of 3 cm. Serial images were coded into the US system and saved on a memory stick.

The investigator evaluated the images at the end of every “experiment day” by using “MyLabDesk” software exportable to computers, which allowed the investigator to assess the thickness of 126 individuals m. masseter. The thickness, in mm. was calculated as the distance between the bright white underlying ramus and the superficial surface of the muscle as showed in Figure 5. Standard settings, anatomical landmarks and “agreement of region of interest and thickness” are accurately described in the protocol drew up by Dr. Lenie van den Engel-Hoek, Dr. N. van Alfen, and Dr. S. Pillen (Appendix IV).

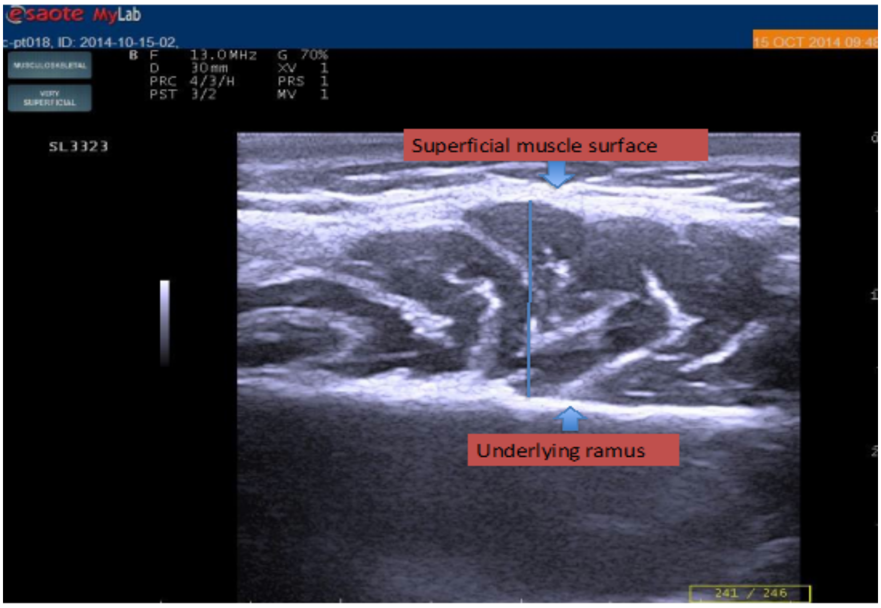

Figure 5. Straight blue line indicates distance between superficial muscle surface and underlying ramus of masseter muscle in its thickest point.

2.4 Experiment' specifications

This is part of a larger experiment, which implies seven examiners (four physiotherapists and three speech therapists) and more measuring tools, however this method section only focuses on the parts that concern the observation of a correlation between MBF and m. masseter thickness investigated by US technology. The protocol of the whole experimental procedure can be found in Appendix III.

Each participant undergoing the whole experiment took about an hour to complete the assessment. By the second experimental week the time estimated for participants decreased to 45 minutes for the whole testing procedure. This was due to the fact that the investigator became more familiar with the US leading to a short but still efficient assessment of participants. The testing was carried out for a total of three weeks.

2.5 Statistical analysis

The data gathered during the experiment will be collected in Microsoft Excel and analyzed with IMB SPSS statistics, version 21.

Demographics such as gender, age, height, weight and other measurements, MBF and US (for the latter contracted and relaxed conditions of m. masseter individually) were analyzed by descriptive statistics.

This data are presented as mean (M), maximum, minimum and standard deviation (SD).

To assess the distribution of MBF (in kg) and m. masseter thickness (in mm.) in both relaxed and contracted states, Shapiro-Wilk test was chosen as normality test (normal distribution→$P > .05$.

Since MBF is only available as functional outcome, we had to compare the two different variables of the US measurements: thickness of m. masseter in a contracted versus the thickness of the same muscle in a relaxed state. These two specific means were compared to each other by using the paired T-test. Significant difference in between means was determined with $P < .05$.

The sample consisted of a heterogeneous population, therefore inter-gender difference for both BF and muscle thickness, was analyzed with independent T-test.

The final objective of the study was to find the correlation (r =) between MBF, therefore the mean of three ultrasound measurements (in contracted state) and the mean of three MBF measurements were used as variables in the correlation analysis. Pearson' s correlation test was used to determine if these two variables were linearly related to each other.

Connection between these two specific quantitative variables can be:

Maximal positive correlation→ r = 1

Maximal negative correlation → r = -1

No correlation → r = 0

The statistical significant difference value will be defined as p < .05.

3 Results

General characteristics of the group of the study participants (59 males and 67 females) such as age, height and weight are presented in Table 1. The mean age of the whole population, with no gender discrimination was 25.1±4.8 years, mean height was 174.1±9.7 cm. and mean weight was 73±17 kg.

Table 1. General characteristics of the participants

		Minimum	Maximum	Mean	SD
Age (years)	Tot.	20,0	40,0	25,1	4,8
	Males	20,0	40,0	26,3	5,3
	Females	20,0	39,0	24,1	4,1
Height (cm.)	Tot.	154	197	174,1	9,7
	Males	165,0	197,0	181,9	6,8
	Females	154,0	182,0	167,2	5,8
Weight (kg.)	Tot.	47	133	73,1	16,5
	Males	62,6	133,0	83,3	17,2
	Females	47,2	86,9	64,1	9,3

SD: standard deviation

The mean and SD of the thickness of the masseter muscle (TMm) and maximal bite force (MBF) measurements are shown in Table 2.

MBF and TMm were always greater in males than in females. Significant inter-gender difference (*P*< .05) was observed for both TMm (contracted→ mean difference= 2.3mm; and relaxed→ mean difference= 2,4mm) and MBF measurements (mean difference= 5,6kg) when performing the independent T-test.

In Table 3 the mean difference in muscle thickness between contracted and relaxed state, as well as between the left and the right side are shown. The outcome shows that there was no statistical difference between right and left m. masseter, but significant difference (*P*< .05) was observed between contracted and relaxed measurements with a mean difference of 1,2mm. Figure 6 shows through means of US images the TMm difference in between conditions and sides.

As mentioned in the method section of this study, each participant started the test either with the US investigation or with the MBF one. An independent T-test showed that there was no statistical difference between individuals starting with US (number of observation (N)= 68) and those starting with MBF (N= 58) assessment *P> .05.*

In order to analyze the correlation between TMm and MBF, normal distribution of those continuous variables was tested by Shapiro-Wilk test of normality. Muscle thicknesses in both conditions and sides and MBF magnitude measurements are normally distributed (*P > .05).*

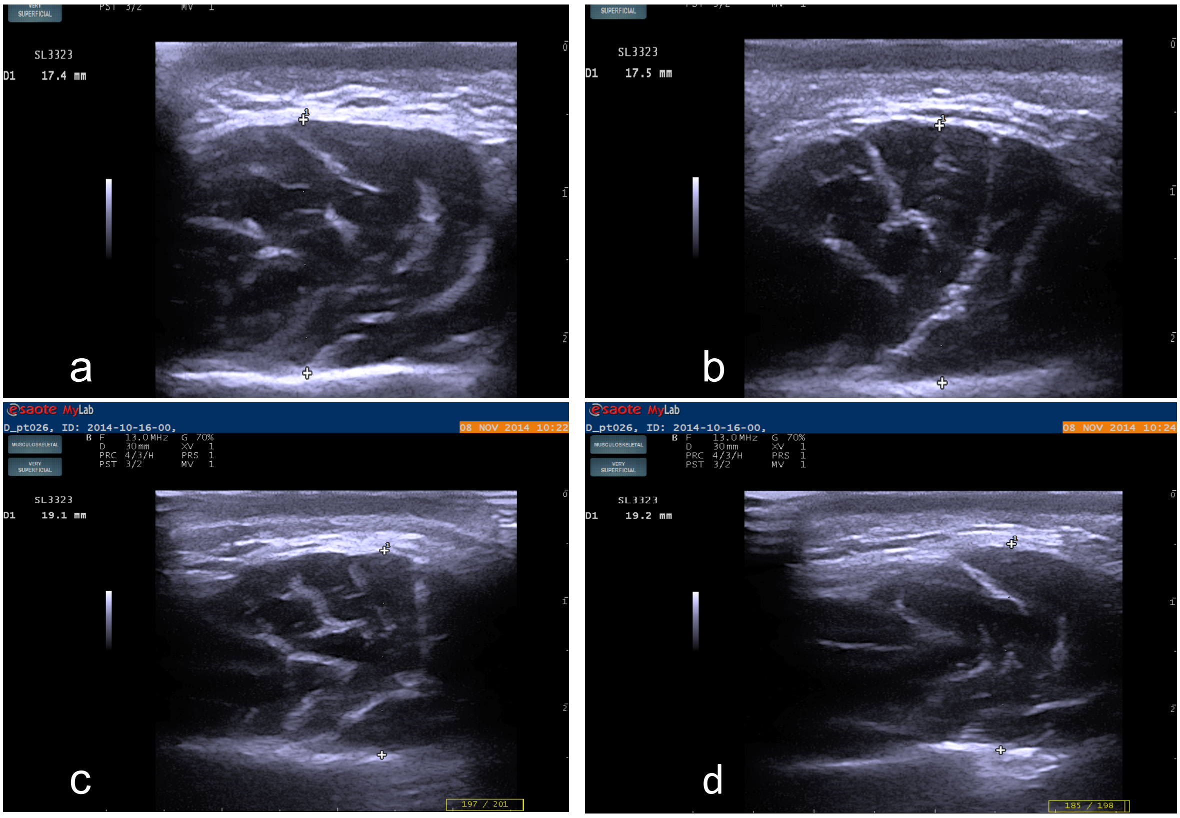

Figure 6. (a) Image of the left masseter muscle in a relaxed state, thickness reported in millimeter. (b) image of the right masseter muscle in a relaxed state; n.b. for this participant 0.1 difference was observed. Overall difference between right and left m. masseter was significant. (c) Image left masseter in a contracted state. (d) Image right masseter in a contracted state, n.b. for this participant 0.1 difference was observed. Nevertheless, comparing the mean of right and left m. masseter in a contracted state there was no significant difference observed.

Table 2. Descriptive statistics of muscle masseter thickness in mm.(per condition and side), bite force in kg. and standard deviation.

		Mean	SD
***TMm. Relaxed**	Tot.	13,6	2,0
	Males	14,8	1,6
	Females	12,4	1,6
***TMm. Contracted**	Tot.	14,7	2,1
	Males	16,0	1,7
	Females	13,6	1,8
***TMm. Right**	Tot.	14,1	2,1
	Males	15,4	1,6
	Females	12,9	1,8
***TMm Left**	Tot.	14,2	2,2
	Males	15,5	1,8
	Females	13,2	1,8
MBF	Tot.	26,8	9,9
	Males	29,8	11,2

Females	24,2	7,8

*TMm: Thickness Masseter muscle.

Table 3. Mean difference between the relaxed and contracted conditions and left and right sides of muscle Masseter thickness in mm.

	Mean Difference	**SD**
TMm Contracted Vs. TMm Relaxed	1,2	0,1
TMm Left vs. TMm Right	0,2	0,1

Pearson test was performed to analyze the correlation between the two methods, US and BF. There was a poor positive correlation between TMm and MBF r= .3, *P< .05*.

The correlation is analyzed by using the values of TMm in a contracted state instead of the relaxed one. This choice is supported by the fact that MBF can only be measured when muscles exert power into an isometric bite, therefore MBF cannot be assessed when the masticatory muscles are relaxed.

The correlation between BF and TMm (contracted) is represented graphically by a scatter diagram with a fit line in Figure 7.

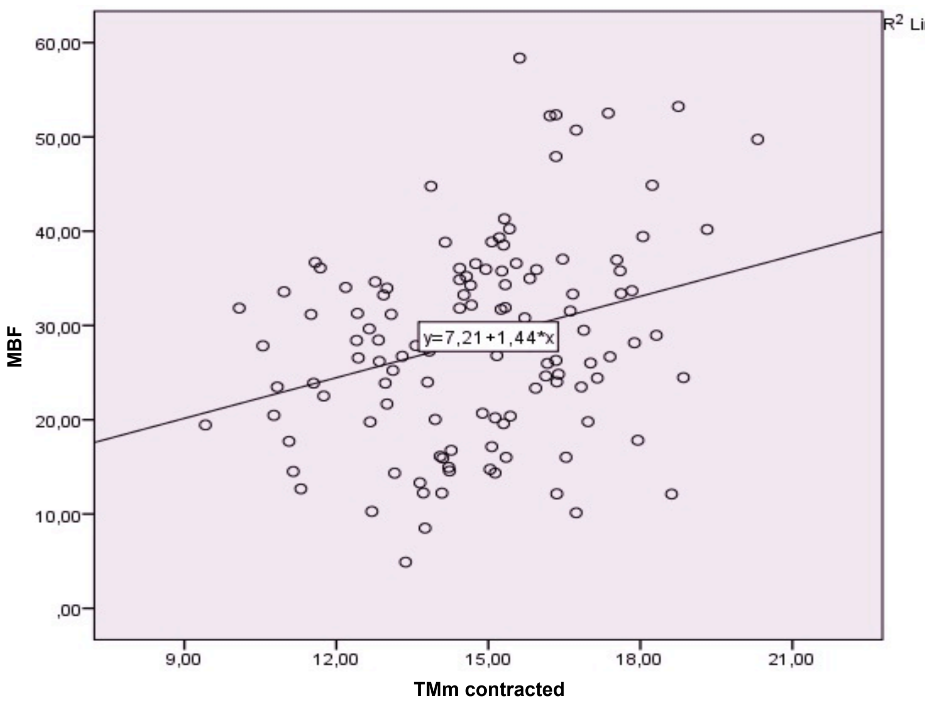

Figure 7. Scatter plot – correlation MBF and TMm contracted

Table 4 shows that when the MBF and Contracted TMm was analyzed with intra-gender discrimination, the correlation between male MBF and male TMm contracted would still be poor r= .2. A similar outcome was found when correlating females bite force with their muscle masseter thickness r= .1.

Table 4. Intra-gender correlation MBF and contracted masseter muscle (TMm)

	Correlation coefficient	P - Value
MBF – TMm contracted Females	.1	0,03*
MBF – TMm contracted Males	.2	0,08 Ns

Ns = not significant (*P>.05), *P< .05*

4 Discussion

In the present study on 126 healthy adults (59 males and 67 females), the correlation between bite force (BF) assessed by the bite force gauge and m. masseter thickness, evaluated by ultrasound (US), was observed and analyzed. Inter-gender difference for both methods, was also evaluated.
When data were pooled for analysis, with no gender discrimination, to evaluate the relation between BF and m. thickness, the Pearson correlation coefficient revealed a poor but still statistically significant correlation between m. masseter thickness and BF ($r = .3$).

Additionally, an independent T- test showed a consistent inter-gender difference ($P < .05$) in both BF (males: 29,8kg; females: 24,2kg) and thickness of the m. masseter in a contracted state (males: 16mm.; females: 13,6mm.).
Due to the large inter gender difference in both BF and m. masseter thickness, a more meticulous intra-gender correlation analysis was performed.
The correlation between males' bite force and males muscle masseter' s thickness was even poorer than the overall correlation ($r= .2$) and it was not statistically significant ($P= .08$). Similar outcomes were found when correlating females bite force with their muscle masseter thickness ($r= .1$; $P= .3$).
Evidence about stronger male bite force and thicker male m. masseter, has been previously shown in other studies. (7,9,11,24,25) This could be attributed to anatomical and physiological differences. Men masseter muscle have fibers type II which produce more force and contract faster compared to type I fibers in females' masseter muscle. (9,12)

Concerning only the US measurements of m. masseter thickness the differences between contracted and relaxed state and left and right mm. masseter were also investigated.
When the mean of 6 left measurements (3 trials of contracted and 3 trials of relaxed) was compared to the mean of 6 right measurements, by using a paired T-test, we found no significant difference between the 2 sides ($P = 0{,}055$). When a more meticulous comparison between left and right in both m. masseter conditions was used we observed that there was still no statistically significant difference in between contracted left/right mm. masseter, but significant difference was observed between relaxed left/right mm. masseter ($P=0{,}03$). This outcome was presented also in one previous study (20) where the right relaxed masseter was smaller than the left relaxed masseter. Although this is statistically relevant, it did not influence our correlation analysis. Bite force is only available when muscles perform contraction; therefore we decided to compare it to the outcome measurements of m. masseter thickness in a contracted state.
This was similarly done by other researchers, who compared only relaxed masseter muscle thickness assessed by US to the thickness of the same relaxed muscle investigated by magnetic resonance imaging, or MRI (only available for the relaxed condition of a muscle). (23)

Although, few previous studies investigating on the association between BF and masticatory muscles, particularly m. masseter and m temporalis, showed that the magnitude of bite force strongly correlates to the size of masticatory muscles (11,22), our study presents a poor correlation between the two variables. The reason of this controversial outcome may be the result of experimental settings differences. Other authors stated that bite force was always measured in between molars and US often assessed the cross-sectional area, which represents the perimeter of the whole muscle. Besides, in our study, only m. masseter was assessed with US, leaving out the temporalis muscle because of time constrains.

The temporalis muscle is mainly active during mastication (molar contact) and isometric molar bite, while only m. masseter with the pterygoid muscle, are active during isometric incisor bite. (26) Isometric incisor bite was, in fact, the way BF was assessed with all the 126 participants. This leads to the conclusion that BF should have been assessed in between first molars or that both m. masseter and m. temporalis' thicknesses should have been investigated.

This could explain why there was no significant difference between the participants who started the testing procedure with the bite force station and the ones starting with US one. When analyzing difference of m. masseter thickness or bite force between this two groups we expected there would be a relevant difference, due to the fact that participants going first through US or BF measurements would be always more tired for the second following assessment. This surprising finding can be associated with the fact that diverse muscles executed diverse types of bite (isometric clenching on molars and isometric incisor bite) (26,27), placing effort on different muscles and that incisor bite produces a weaker force than the molar one. (22)

It was mentioned that there is a thickness' significant difference related to the relaxed m. masseter outcome measurements. One previous study from 2004, researching the effect of transducer' position on m. masseter thickness, has shown that "spatial position of the transducer has a clear effect on muscle thickness" (P= 0,0001). (28)

In study like this, it is very hard to understand if the difference is due to individual parameters' differences assessed during experiment or to the method measurement error (which is the difference between measurement one and measurement two expressed in percentage). (23)

We tried to reduce the individual parameters differences by standardizing the US testing procedure. Standardization was inherent to location of the transducer, sitting position, and guidance though the whole assessment (participants were asked to relax by keeping lips contact and then contract by forcefully assuming an intercuspal position).

One thing that was not standardized during US assessment was the resting time in between trials and lack of m. masseter activity before their BF and muscle thickness was assessed; participants were not worn to avoid eating or just simply chewing gums before being assessed, which proved to interfere with the results in one other similar study. (25)

Additionally, the method measurement error was not calculated in this study, which means that the percentage of relaxed and of the contracted measurement' s error has not been stated.

According to previous literature about correlation between m. masseter thickness, assessed by US and by other methods, lower reproducibility of the relaxed measurement of m. masseter than the contracted one is more likely to happen. In fact, the method error using US to assess m. masseter thickness was always greater for the relaxed measurements than for the contracted ones. (23–25) This could be due to the fact the even the slightest pressure might reduce the thickness of the muscle, which is not likely to happens if the muscle is contracted since it is more difficult to squeeze it.

Standardization of morphological features was not achieved. Although exclusion and inclusion criteria of a study can surely help the results to be more homogeneous, we decided to not measure morphological differences in between individuals since it was not strictly inherent to our research question. Nonetheless, inter-gender difference in m. masseter thickness and bite force outcomes was great; this significant difference underlines the fact that morphological features have, obviously, an influence on muscle thickness and consequentially on bite force.

The influence of morphological features such as cranio-facial dimension, dental arch width, occlusal contact, tooth size, mouth ROM have widely been investigated when correlated with BF or jaw muscle size, reporting high degree of association. (7,9,10,22,25)
For instance, men have wider dental arch, larger teeth and greater ROM, which all together produced higher clenching force. (7–9) Additionally there is some evidence the m. masseter size and BF negatively correlated to large anterior vertical cranio-facial dimension and positively to the large posterior vertical one. (22)

Furthermore, morphological features might be modified by the presence of complaints or diseases. Recently some authors have started to research more deeply into how the quality of mastication is directly associated to cognition impairments and how does dementia effect the function of masticatory muscle. (3,4,30) In some studies using PET autoradiography or functional MRI it has been shown that mastication improves cerebral circulation (3,25) working a muscle pump. People with the dementia do not efficiently use this “muscle pump”. This is due to fact dementia is related to aging and aging is associated with chewing muscle degeneration, loss of teeth, and poor nutrition. (2–4)

US has been showed to be an efficient and quick diagnostic tool, which evaluates morphological characteristics and changes in real time display and allows the investigator to have immediate sides’ differences. It is patient-friendly and suitable for all the ages, especially when compared to the BFG.
More attention is being dedicated to the US technology as diagnostic tool into praxis. At the moment US is mainly used for research purposes, f.e. to measure masseter muscle thickness and its quality and the quality of other muscles in patients with amyotrophic lateral sclerosis (ALS). Nonetheless, the use of US for clinical purposes is gaining importance. Therefore further research is needed, particularly to observe how directly related are mastication and cognition.
It is recommended to choose an older population (>40). It is known that BF stays fairly constant in between this range (9,10) and to extend the research to people with complaints to see what changes occurs in jaw muscles size and on the magnitude of bite force.

A qualitative and quantitative study could also be carried out to empower the accuracy of the investigation. Since aging is related to deterioration of muscle and cognition (2,3,30) and poorer mastication is associated to lower food intake (4) questionnaires about nutrition, for instance, could be used to assess the correlation between diets and quality of the muscles. This could greatly help physiotherapist to adopt a very specific

preventive approach to the problem by building up masticatory muscle training, by chewing on hard elements for instance.
Additionally, we recommend future researchers, continuing our experiment on different samples, to make sure that the measuring tools' protocols are accurately drawn up and that muscle thickness and BF will be assessed in the most similar way, having the participants to execute the same type of bite when being assessed with the bite force gauge and the ultrasound technology. Supposed that the research will be continued by assessing the temporalis muscle thickness, the probe of the BFG must be placed in between the molars to assure full activity of the temporalis muscle, which has been shown to be the most active when biting on the molars, and relatively quiet during the isometric incisor bite. (26)

Another interesting fact is that cranio-facial dimension does not only differ in between genders, but it also varies in between different ethnicities. Knowledge about how different ethnicities effect bite force and jaw muscle size is poor. It is also not clear yet on what degree different factors such as nutrition, climate, socio-economic status influence intra- and inter-ethnicity differences. Therefore, more investigation about this broad topic is required.

Although this study shows a poor correlation, it is important to highlight the impact that such a large sample (N= 126) could have on the correlation coefficients. (31) It is common for a correlation to decrease as the sample increases. In fact, it has been observed that larger correlation coefficients are often found with smaller samples', suggesting that the correlation coefficient may be overestimated.
The true correlation between US and MBF will be only the result of the meta-analysis of all the studies about this topic.

5 Conclusion

Considering the above discussion, we can conclude that poor correlation between bite force and m. muscle thickness was found.

Despite of the limitation of this study there is hope that this outcomes will open more doors to further research into the field of mastication and oral care and to a new ideal physiotherapeutic approach which might bring geriatric clinics to implement preventive programs for masticatory muscles and to utilize US technology in the working framework, as an efficient diagnostic tool in the investigation of masticatory muscles' morphological change.

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7 Appendices

Appendix I: Participation letter

Dear Sir or Madam,

We would like to ask you to participate in a research of training Speech-Language and Physiotherapy Therapy at Fontys Paramedische Hogeschool in Eindhoven. Whether you want to join is completely up to you. Before you decide, it is important to inform yourself about the research. Please read the newsletter thoroughly and discuss it with your partner, friends or family. If you have any questions after reading, feel free to contact the researchers. You can find the contact information below.

Aim of the research
Speech-language therapists help people with speech, language, voice, hearing, chewing and/or swallowing problems. This research focuses on the chewing problems and problems with the muscles in the mouth. In order to treat these problems properly, speech-language therapists have to know the strength of the muscles. However, it is unknown how reliable the instruments used to test these strengths are. In this research, we will test the reliability of these instruments.

Physical therapists help people with problems in their entire musculoskeletal system, such as the muscles, tendons and joints. A large amount of people have to deal with masticatory muscle problems or temporomandibular joint problems at some point in their lives, which can subsequently lead to problems in jaw, head and neck as well. Physical therapists need reliable instruments in order to make accurate diagnosis.

Conditions for participation in the research
- You're between 20 and 40 years old and understand the English language.
- You haven't had jaw- and/or masticatory muscle (pain when chewing, pain on the jaw), hand, wrist, forearm or elbow complaints in the past six months.
- You don't have a medical history regarding jaw- and/or masticatory muscle, hand, wrist, forearm or elbow surgery.
- You're not having any complaints or pain during the measurement.

Subject matter of the research
Four measurement instruments will be examined, namely:
1) Bite force meter
2) Myoscanner
3) Echo
4) Hand grip force

How is the survey conducted?
Bite force meter
This test requires the participant to bite on a sterile stick which will measure the strength of the bite. You will have to bite a total of three times.

Myoscanner
A plate is placed on the cheek, while you bite your back teeth. The plate then measures the strength of the bite. This is done three times per side, making a total of six bites. Your lip- and tongue strength will also be measured three times, these results are for further research in the future.

Echo
A small echo-scanner is placed on the cheek to measure the composition of your chewing muscles.

Hand grip force
The participant will be asked to hold onto a hand held dynamometer and close the fists firmly to measure the hand grip force. Two measurements will be taken of each hand.

Reliability

In order to test reliability, all tests will have to be done two times.

Weight and height measurements
To put data into context we will measure your weight and height and additionally we would like you to fill in a questionnaire regarding your teeth, eating habits and any relevant complaints you might have. Please note that your answers will be handled discretely and anonymously.

Please note that the entire research will be free from pain and discomfort.

What is expected from you?
We invite you to come to the Fontys TF building in Eindhoven. There you will have to fill in a questionnaire and a consent form. After these, the measurements will take place.

What are the possible advantages and disadvantages of this research?
There are no physical advantages or disadvantages. The measurements are free from pain or discomfort. It will, however, take up about one hour of your time.

However, physiotherapists and speech-language therapists may benefit greatly from the gathered data which can possibly improve future treatments in both fields.

What happens if you do not wish to participate in this survey?
Participation is completely voluntary. You decide whether you participate in the research. If you choose not to participate, you do not have to do anything or give an explanation. We would thank you for your attention and look for other participants instead.

Even when you do join, you can always stop at any point without giving a reason.

What happens to your data?
All data is anonymous and will be used for medical purposes only. However, we would like to save the data even after the research, to be used for other researches or revision of the current research. Please note that this too will be done anonymously, which means your name cannot and will not be linked to your data.

If you do not want the data to be stored, you can indicate your choice on the consent form.
If you want to receive the results of the research, please leave your e-mail address on the form.

Is there a fee if you participate in this research?
You will get no money for this research.

Which medical ethics committee approved this study?
This research was approved by the Fontys Ethics Committee.

More questions?
If you have any more questions, feel free to contact one of the researchers. Contact details are below. If you want to participate, we ask that you sign the consent form (see Appendix) and take it with you on the day of the research. Thank you.

Yours sincerely,

The researchers
Students Speech-Language therapy: Renske Damen, Michelle de Poot, Sanne van de Schoor.
Students Physical therapy: Margot Gabriele Falcone, Virpi Rajala, Robin Riessbacher, Joery van Rooijen.
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Appendix II: Informed consent

Certificate of consent:

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print Name of Participant ____
Signature of Participant ____
Date ____________________________Day/month/year of Consent

Appendix III: Measurement protocol

Protocol use myocanner 160-B version Helderop and Verlinden

Step plan	**Explanation**
Instalation	• Install Program Myocom VSI on the computer. It will make use of the accompanying CD. • Install interface. For Windows Vista and Windows 7 (the drivers must be installed): • Make sure you are connected to the Internet. • Connect only the interface (black connector with USB connector and serial port connector) to the computer. • It opens a window with: new hardware found • www.OMFT.info let the computer on the Internet self-search for drivers • Multiple drivers will be installed. After some time, a new window will appear: Harware installed and can be used.
Connecting Cables	• Connect the footpedal to FOOT in the Myoscanner. • Connect the probe to PROB (probe) in the Myoscanner. • Connect the newly installed Manhattan interface cable to the long black computer cable, and then one side of the extended cable into the USB port of the computer and the other end in COMP in the Myoscanner.
Start using the myoscanner	• Start Myocom. • Set the Myoscanner on by pressing the ON / OFF button.

Protocol measurements myoscanner 160-B version Helderop and Verlinden

Cleaning	• The researchers clean their hands with a disinfecting agent. • The probe and the masseter plate gets cleaned with a tissue with alcohol.
Preperation of measurements m.masseter	• Instruction to participant: "I'm going to measure how strong your bite muscles are. We will use the Myoscanner, this device. I will now put a dot on your cheek so I can measure the point where the muscle is the thickest. • Place the masseter plate in the probe and ensure that it properly sits in the end of the slot. • Place four fingers together on the m. Masseter and let the participant tightly bite and try to feel for the bump (= the thickest

	part) of the m. Masseter • Go with one finger slowly over the area looking for the site of the biggest bulge. • Mark this spot with a dot with an eye pencil. • Mark the middle part of the probe (rectangle) with a dot with eye pencil. • Place the two dots exactly on each other.
Measurements m. masseter	• Instruction participant: "I will put the plate against your cheek, and then ask you to bite as hard as possible. We will do this several times. I will tell you when you can bite. • Stand behind the participant. • Turn the head of the participant horizontally sideways as well and back slightly with the nose up a little. • Stabilize the participant against your chest. • Lightly put your arm on the head of the participant so you are able to reach the masseter plate with two fingers. This ensures a good stabilization against the ear of the participant. In this stage of the test the probe is not held yet against the skin, thus against the masseter. • Hold the probe to the black knob: middle and index finger under the button, the thumb on the button. Put the probe gently on the masseter body (marked dot). • Wait until a slight jittering on the Myoscanner is visible, press the foot pedal, allowing the scanner reset to ZERO. • Now provides the participant the instruction 'bite the teeth firmly together and bite as hard as possible' and remove immediately after the measurement the probe with the masseter plate from the cheek. • The researcher indicates when the participant may stop biting. • Stay in the same place and rotate the head so that now the other cheek is visible with the other m. Masseter. Again, fix the head against your chest. Repeat the above steps, where you fixate the plate with two fingers against the ear and later place the probe slowly against the cheek to start the measurements. • Three measurement procedures will be performed per side. • The dot on the face is removed with makeup remover. • The result is the average of three correct measurements, measured in pounds.

Based on the instructions of the Myoscanner from B. Verlinden and P. Helderop

Protocol for use of the VU Bite Force Gauge

Step Plan	Explanation
Start using bite force guage	• Attach the mouth piece to the meter. • Set the bite force meter on by pressing the ON / OFF button.
Measurements	• Insert a latex-free protection on the mouthpiece (the probe). • Place the mouthpiece between the teeth of the participant. • Check if the upper and lower teeth are properly on the mouth piece. • instructed the participant to bite as hard as possible.
Results	• The 'peak' is noted. • Wait until you hear a beep (pause to rest) and note the recalculated value (also in PEAK). • Then press 'ZERO' and "PEAK" simultaneously (returns to 0), and then measure again. • It is measured a total of three times. The result is the highest value of three measurements, measured in kilograms.

Protocol measurements VU Bite Forge Gauge

Step Plan	Explanation
Cleaning	• The researchers clean their hands with a disinfecting agent.
Explaining the measurements	• Patient Instruction: "We want to know how hard you can bite. For this, we will use the bite force meter, this device. I will put it between your teeth and ask you to bite as hard as possible. We will do this three times. In between we take a short break."
Measurements	• Set the bite force meter and place a latex-free protective cover over the mouthpiece (the probe). • Place the mouthpiece between the teeth of the participant. • Check if the upper and lower teeth are in a good place on the mouth piece. • Now give the participant the command to bite as hard as possible: "Now you may bite as hard as possible until I say stop." • Encourage the participant to "bite, bite, bite ..." • Remove the probe from the mouth. • Note the "PEAK" after the beep. • In total, there will be three measurements. The result is the highest value of the three measurements, measured in kilograms.

Based on the protocol of the bite force meter as described in the unpublished thesis 'Maximum bijtkrachtmeting' from 2014 M. van Buren & L. van Ast.

Protocol measurements ESAOTE My Lab One with 13 Mhz transducer

Step Plan	**Explanation**
Start using echosystem	• Connect the transducer to the display. • Set the ESAOTE My Lab One by pressing the ON / OFF button.
Instructions to Participant	• If necessary, a brief introduction to ultrasound • Information about the order and quantity measurement • Information on the biting intensity during the contracted measurements. • Information about the bite force during contracted measurements. • Information about relaxation during relaxed measurements
Measurements	• Place gel on the transducer • Place the transducer transverse to the m. Masseter of the participant. • Place the transducer with the marker pointing towards the ear. • Follow the jawline. • Rear section towards the earlobe. • Measure both left and right. • Measure both relaxed and contracted.
Results	• Pressing on "FREEZE" will screenshot the muscle. • The thickest measurement will be taken from the thickest part of the muscle perpendicular to the line of the mandible. • The measurement is taken from the inner side of the muscle, fascia not counted, up to the inside of the muscle on the mandible side. • Here after, the transducer will get lifted off the skin and the measurement starts again until the tester has three relaxed and three tightened m. Masseter measurements. • 24 measurements per researcher, 48 measurements in total. • The measurements are in millimeters.
Cleaning	• The researchers clean the transducer after each participant with a paper tissue.
Sequence	• The participant first goes to "researcher 1," then to "researcher 2" then back to "researcher 1" and finally back to "researcher 2."

**To assess m. Masseter, the protocol from Dr. Lenie van den Engel-Hoek, Dr. N. van Alfen, en Dr. S. Pillen from the Radboud UMC has been used.*

Protocol measurements Jamar Hand held dynamometer

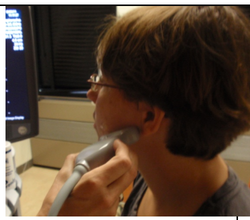
Step Plan	**Explanation**

Preperation for measurements	• For carrying out the measurement, a hand grip strength meter (JAMAR), a seat, and a table are required. • The participant sits on a chair with the arm resting on a table with the elbow at a right angle. After each measurement, the pointer of the meter must be turned back to zero.
Demonstration and practice	• First show the participant how to grip the strength meter and how to squeeze (as hard as possible). • Let the participant try the strength meter by lightly squeezing with the right and left hand. • Adjust the position of the handle so that it feels comfortable in the participants hand. The distal joint of the middle finger should run parallel with the palm of the hand. The middle joint of the middle finger should be at precisely 90°.
Execution of hand grip measurement	• The participant squeezes the handle as hard as possible for approximately two seconds. • Make sure that the dial is facing outwards. • Read the measurement result and round it off to one kilogram. • Perform the test twice in each hand (if possible) and record the highest value. • Always let the participants take 15 – 20 seconds rest in between.

Based on the protocol from the JAMAR Manual for Hand Grip Strength Meter

Appendix IV Protocol Ultrasound measurements

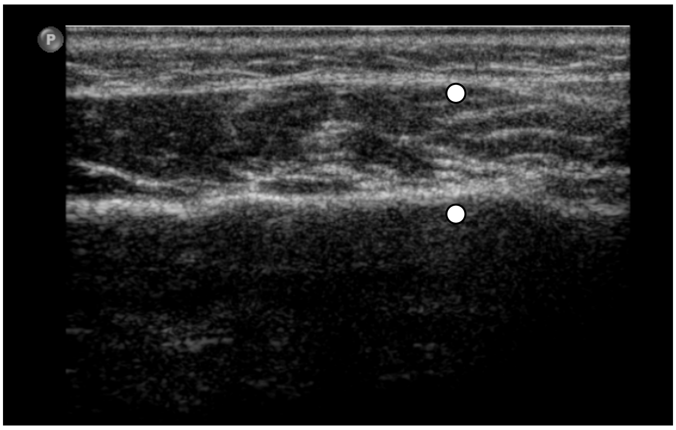
›rotocol spierogrefie van m. Masseter

I. masseter (probe L10-5) .andachtspunten: Iarkering op de probe chting het oor plaatsen .aaklijn volgend, laag langs e kaak .chterste gedeelte bij oorlel inks en rechts .eeld: ı principe moet de kaaklijn orizontaal liggen en meet je e dikte loodrecht op de pieroppervlakken	Preset = C Spierecho (G78/E0/100 %) 8.5MHz, 4 cm		 	**Analyse** **Spierdikte m. masseter** **Echo intensiteit m. masseter** **Gemiddelde van drie metingen**

ıfspraken ROI en dikte metingen:

PIER	ROI	DIKTE
Iasseter	ROI zo groot / breed mogelijk, niet de zijkanten van de opname; niet de fascie aan de buitenkant van de spier meenemen, wel eventueel IN de spier (gemiddelde van drie metingen)	Muscle size : loodrecht op onderste lijn (= kaaklijn, moet horizontaal zijn), zie C. Breedste gedeelte. (gemiddelde van drie metingen)
emporalis	ROI zo groot / breed mogelijk, niet de zijkanten van de opname; niet de fascie aan de boven /buitenkant van de spier meenemen, wel eventueel IN de spier; bovenkant zit veel bindweefsel, niet meenemen (gemiddelde van drie metingen)	Muscle size : loodrecht op bovenste lijn, horizontaal, op het diepste punt, niet de fascie / bindweefsel aan de bovenkant meenemen, zie D. (gemiddelde van drie metingen)

A. Masseter



Muscle size: onderlijn (= kaaklijn), breedste gedeelte (fascie tegen bovenkant = meetpunt 1 ; onderkant is = meetpunt 2)
ROI: binnen facie lijnen, niet buitenste 1 cm van de afbeelding.