Assessment of the echogenicity of the masseter and temporalis muscle.

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march 2016 –

Version 1.0
Preface

This study was done at Fontys University of Applied Sciences in Eindhoven. The research process started in February 2016 and ended in June 2016. In this time, I was fully dedicated to this study working closely together with Guilemette Ricour.

I want to take this opportunity to thank all the people who supported and helped me during this process. First of all, I want to thank Daniel van Leeuwen who was always available for help and for his guidance during the process. Secondly, I want to thank Guilemette Ricour, we worked together on our thesis and she helped me a lot during the experiment and afterwards. Additionally, I want to thank Marc Schmitz who made me enthusiastic about the topic and taught me how to use the musculoskeletal ultrasound device. I want to thank Marcos Fazenda and ESAOTE as well. They made sure we could use their device, which made it possible we could investigate more people in the same timeframe.

Last but not least I want to thank my family, friends and boyfriend, during the period of writing my thesis I went through a hard personal time and they made sure I made it through.

Hanneke van der Steen,
Gemonde, June 2016
Abstract

**Introduction:** Risks for stroke or developing dementia are inversely related to masticatory function. Musculoskeletal ultrasound (MSU) can be used to assess masticatory muscles. By measuring the echogenicity, quality of muscles can be determined. Therefore, echogenicity can give information about a muscle's composition. The aim of this study was to assess the echogenicity of the Masseter and Temporalis muscles and the influence of Body Mass Index (BMI), sex and preferred chewing side.

**Method:** 102 participants in the age range between 20 and 26 were measured. Images of Masseter and Temporalis muscles were taken and preferred chewing side was noticed. To determine the echogenicity, the images were analysed with ImageJ. Statistical analysis was done with SPSS.

**Results:** 102 participants were measured (53 women, 49 men), with 2 devices, of which device 1 was used for assessing norm values. Mean echogenicity (N=43) of device 1 of the Masseter muscle in women was 82.4±11.68 and in men 70.4±10.97. The mean echogenicity of the Temporalis muscle was for women 81.7±14.21 and for men 74.7±10.97. Compared to men (N=17, M=67.5, SD=121.98) women (N=26, M=81.3, SD=12.70) had significant higher echogenicity of the Masseter muscle on the preferred chewing side (p-value=0.001). For the non-preferred chewing there was no significant difference found but a trend was found (p-value=0.058). For the Masseter muscle (N=43) there was a significant difference found between preferred (M=75.9, SD=14.38) and non-preferred (M=80.1, SD=14.55) chewing side (p-value=0.048). For the Temporalis this was not found (p-value=0.359).

**Conclusion:** The echogenicity of the masticatory muscles was higher in women compared to men. For the Masseter muscle there was a significant difference between preferred and non-preferred chewing side with N=43. Taken the complete participation population (N=102) no significant differences were found between preferred and non-preferred chewing side in both Masseter and Temporalis muscles.
Samenvatting

**Introductie:** Risico's voor het krijgen van een beroerte of voor het ontwikkelen van dementia is evenredig gerelateerd aan de kauwfunctie. Musculoskeletal ultrageluid (MSU) kan worden gebruikt om de kauwspieren te beoordelen. Door het meten van de echogeniciteit kan de kwaliteit van een spier worden bepaald. Dit geeft informatie over de samenstelling van een spier. Het doel van deze studie was het beoordelen van de echogeniciteit van de Masseter en Temporalis spieren en de invloed van Body Mass Index (BMI), geslacht en voorkeurskauwzijde.

**Methode:** 102 participanten in de leeftijd van 20 tot 26 jaar zijn gemeten. Afbeeldingen van de Masseter en Temporalis spieren werden genomen, ook werd er gevraagd naar voorkeurskauwzijde. Voor het bepalen van de echogeniciteit, de afbeeldingen werden geanalyseerd met ImageJ. Statistische analyse werd gedaan in SPSS.

**Resultaten:** 102 participanten zijn gemeten (53 vrouwen, 49 mannen), met twee apparaten, waarvan device 1 werd gebruikt om referentiewaarden te bepalen. Echogeniciteit gemiddelde (N=43) van Device 1 van de Masseter spier bij vrouwen was 82,4±11,68 en bij mannen 70,4±10,97. De echogeniciteit van de Temporalis spier was voor vrouwen 81,7±14,21 en voor mannen 74,7±10,97. In vergelijking met mannen (N=17, M=67,5, SD=121,98) hadden vrouwen (N= 26, M=81,3, SD=12,70) een significant hogere echogeniciteit van de Masseter spier aan de voorkeurskauwzijde (p-waarde=0,001). Voor de niet-voorkeurskauwzijde werd er geen significant verschil gevonden maar een trend met p-waarde van 0,058. Voor de Masseter spier (N=43) was er een significant verschil tussen de voorkeurskauwzijde (M=75,9, SD=14,38) en niet-voorkeurskauwzijde (M=80,1, SD=14,55) (p-waarde=0.048). In de Temporalis spier is geen significant verschil gevonden (p-waarde=0.359).

**Conclusie:** De echogeniciteit van de kauwspieren was hoger in vrouwen in vergelijking met mannen. Voor de Masseter spier is een significant verschil tussen voorkeurskauwzijde en niet-voorkeurskauwzijde met N=43. In de gehele participanten populatie werden geen significante verschillen gevonden tussen voorkeurskauwzijde en niet-voorkeurskauwzijde voor zowel de Masseter als de Temporalis.
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1. Introduction

In the United States every year nearly 129,000 people are killed as a result of having a stroke. This is the fifth leading cause of death and it is the most common cause of disability in the country. Globally the prevalence of stroke is 33 million and 16.9 million people of these 33 million suffered a stroke for the first time. Stroke is, after heart disease, the second leading cause of death (1). Other data show that dementia is the leading cause of disability and stroke is the second leading cause (2). In 2010 it is estimated that worldwide 35.6 million people have dementia and it is predicted that this number will double every 20 years (3). The increase especially occurs because people have a longer life expectancy and risk factors are increasing. Both weak chewing abilities and low cerebral blood flow are risk factors for either having a stroke or developing dementia (4–7).

A relationship was found between mastication ability and general health, physical fitness, mental status and nutritional status in elderly people (8,9). Gazynska et al. (8) found that the use of medication is higher in elderly people with a weaker Masseter muscle strength and they need more help for their activities of daily life. Other studies have shown significant correlations between mastication, chewing abilities and cognitive function in both humans and animals (5–7). A decreased chewing ability is a risk factor for cognitive impairments, including developing dementia (7,10). Mastication increases the blood flow into parts of the brain which are concerning learning, memory and cognitive skills. The areas involving are the sensorimotor area, the supplementary motor area and the cerebellum and striatum. The increase in blood flow with mastication to these areas are respectively 25-28%, 9-17% and 8-11% (11). Another study found that mastication stimulates parts of the hippocampus as well. This is a part of the brain which is important for learning and memory abilities (10). With aging the brain function slows down. However, with mastication the brain activity can be increased and cognitive impairments could be reduced. Good quality of mastication functions declines the risk of stroke and developing dementia and contributes to a better general health in elderly people.

Mastication occurs primarily in the temporomandibular joint (TMJ) and is induced by the masticatory muscles. The most import masticatory muscles are the Masseter muscle and the Temporalis muscle. The function of the Masseter muscle is to elevate the mandible and thereby closing the jaw. The function of the Temporalis muscle is elevation of the mandible, retraction of the mandible and stabilizing the TMJ. Retraction is bringing the mandible backwards. Partly due to the previously mentioned risk factors it is important to evaluate the masticatory muscles function in elderly people and to monitor their chewing abilities. The Masseter and Temporalis muscles can be evaluated by magnetic resonance imaging (MRI) or musculoskeletal ultrasound (MSU) (12). MSU has the lowest costs and can be used in primary caretaker settings. There is a significant correlation found in measuring the muscle thickness in the Masseter muscle with MRI and with MSU. This tells one that MSU can be used in primary caretaker settings to evaluate the Masseter muscle (12).
Besides measuring the muscle thickness with MSU one can also determine the echogenicity of a muscle. A higher value for the echogenicity indicates that a greater amount of ultrasound is reflected back to the transducer by the tissue or material (13). The increase of non-contractile tissue in muscles results in an increase of the muscle’s echogenicity (14), therefore an increase in white areas on the ultrasound is the result of an increase of non-contractile tissue. This non-contractile tissue consists of intramuscular fat and connective tissue. There has been a significant correlation found between the increasing of the echogenicity of a muscle and the replacement of intramuscular fat, more than the correlation found between echogenicity and the amount of connective tissue (14).

Strasser et al. (15) found an increase of the echogenicity in muscles in elderly people, and in line with this William et al. (16) showed that the percentage of non-contractile tissue increased when a person gets older. With aging, the number and size of muscle fibres decline and they are replaced by intramuscular fat, which leads to changes in the capacity for compression and elasticity of the tissue (3). This implicates that the echogenicity of the masticatory muscles changes. Before one can investigate this change in elderly people with and without chewing difficulties it has to be investigated at a healthy and young population. This has not been done yet for the masticatory muscles.

Variables on which could influence the echogenicity are sex and Body Mass Index (BMI). An earlier study by Caresio et al. (17) showed a difference in the echogenicity of the Biceps Brachii, Rectus Femoris, Vastus Lateralis, Tibialis Anterior and Medial Gastrocnemius muscles between sexes. Women had a higher echogenicity compared to men (17). And Caresio et al. (17) found a relation between the echogenicity of a muscle and the thickness of the subcutaneous fat layer. An increase of subcutaneous layer correlates to an increase of intramuscular fat and an increase of the echogenicity.

Yamasaki et al. found that there is a relation between chewing side and muscle activation (18). Therefore there could be a relation between preferred chewing side and muscle thickness. There is no literature found about the relationship between chewing side preference, muscle thickness and echogenicity of the Masseter and Temporals muscles.

The aim of this study is to investigate the echogenicity of the Masseter muscle and Temporalis muscle in students within the age range between 20 and 25 years old. Furthermore, the influence of sex, BMI and preferred chewing side on the echogenicity will be determined.
2. Method

Study design

This was a quantitative cross-sectional study determining the echogenicity of the Masseter and Temporalis muscles. And secondly, the influences of sex, BMI and chewing side preference on the echogenicity of these muscles were determined. The muscles were scanned with musculoskeletal ultrasound MyLab™ One by ESAOTE with a 13.0 MHz transducer and images were taken from the Masseter and Temporalis muscles. The echogenicity of the muscles was determined from the images and were analysed with a program called 'ImageJ'. BMI was calculated by measuring height in meters (m) and weight in kilograms (kg). Weight was measured with a HEMA type XR-701 scale to the nearest 0.1 kg. Height was measured with a LUFKIN 5m ultralok Y25CM tape against the wall to the nearest 0.01m.

Participants

In this study 102 participants were measured, 49 men and 53 women. Men and women within the age of 20 to 26 years old were included. They were excluded when having jaw and/or teeth pain in the last 6 months. Participants with the inability of understanding and speaking English were excluded as well. Participants were not allowed to eat an hour before they participated in the experiment. When the participants agreed to participate they signed the informed consent form (English appendix IV, Dutch appendix VI).

Data collection

The echogenicity of a muscle was measured by analysing the image taken with MSU (appendix VII). The images were taken with two MyLab™ One by ESAOTE with a 13.0 MHz transducer. Device 1 was in possession of Fontys University of Applied Science and Device 2 was borrowed from ESAOTE Maastricht. The standard settings for the MSU device were set for all participants. These settings for the Masseter muscle were: a gain of 65%, this could be adjusted, if necessary, within the range from 62% till 68%, a frequency of 13.0 MHz and at a depth of 3 cm. For the Temporalis muscle the settings were: a gain of 65% within the same range, a frequency of 13 MHz and a depth of 4 cm. All images were taken with the lights switched off. Two researchers made the ultrasound images. One researcher performed all the tests from one participant. Appendix I shows the flowchart in which order the participants were measured. Besides images of the relaxed muscles, images of the contracted muscle were taken as well. In total there were 12 images made for each muscle.

The researchers were two fourth year students of physiotherapy at Fontys University of Applied Science. The students got familiar with the MSU device in two workshops and an e-learning program by Marc Schmitz and his company SonoSkills. They practiced for 15 hours before starting the experiment. They made an agreement where to put the transducer prior to the start of the experiment.
Before placing the transducer on the correct position, one put a gel on the transducer to completely cover the surface of the transducer to improve the contact between the transducer and the skin. The transducer was placed on the skin with very gentle pressure. As well the transducer was always placed perpendicular to the bone to avoid anisotropy. The light on the transducer was always placed to the left.

For the Masseter muscle the transducer was placed parallel to the jawline, at the same level as the earlobe, shown in figure 1 and 2.

![Figure 1 - Location for the transducer to assess the Masseter muscle](image1)

![Figure 2 – Masseter muscle. Black line shows the place where the transducer is put. From: https://www.kenhub.com/en/library/anatomy/masseter-muscle](image2)

The Temporalis muscle was measured by placing the transducer on the line between the orbit and the top of the ear as well as about one centimetres cranially to the zygomatic arch, shown in figure 3 and 4.

![Figure 3 – Location for the transducer to assess the Temporalis muscle](image3)

![Figure 4– Temporalis muscle. Black line shows the place where the transducer is put. From: https://www.kenhub.com/en/library/anatomy/temporal-muscle](image4)
The images taken by MSU were analysed by a program called 'ImageJ' (appendix VIII). This program produces a grayscale analysis of the Region Of Interest (ROI), which can be manually selected, selected by “rectangular marquee tool” or by “polygon selection”. The intra- and interreliability of all the selection options is high (13). The “polygon selection” was used to determine the echogenicity of the muscle. ROI was used to select on the inner side of the fascia and the superficial part of the bone. For the Masseter muscle the ROI selection is shown in figure 5 and for the Temporalis muscle the ROI selection is visualized in figure 6. The MSU image is in black, white and gray. A muscle is relatively black because it has a low acoustical impedance, which means that it reflects relatively few ultrasound beams back to the transducer. This gives the muscle a low echogenicity (19). The increase of non-contractile tissue in muscles results in an increase of muscle echogenicity (14), the muscle becomes whiter on the ultrasound image (19). Values range from 0 till 225, for which 0 is a low echogenicity and 225 is a high echogenicity.

Participants were asked for preferred chewing side. When the participant was not familiar with their preferred chewing side, they got the opportunity to experience their preferred chewing side after the images were made. In an excel document was kept which device was used per participant.

**Statistical analysis**

The values were processed in IBM SPSS statistics, version 21. First descriptive statistic was used to find averages, median, minimum, maximum and standard deviation of the echogenicity, age, BMI, chewing side preference and sex. The average echogenicity per muscle in relaxed state per side was calculated by averaging the three images that were taken. The average echogenicity of the participant was determined by calculating the average of the six images of the relaxed muscle. The Shapiro-Wilks test was performed on the data to see if the data was normally distributed. The data was normally distributed when p-value > 0.05. When the data was normally distributed a Pearson’s correlation coefficient was calculated. If the data was not normally distributed, Spearman’s rho was calculated. Statistical significant level was set on p-value < 0.05. A strong correlation was set on a Pearson
correlation coefficient higher than 0.80, and a moderate correlation between 0.60 and 0.80. A weak correlation was set on a coefficient lower than 0.60. The relation between echogenicity and sex was determined by paired t-test. The relation between the echogenicity of the preferred chewing side and the non-preferred chewing side was determined by a paired t-test. The Intra Class Coefficient (ICC) was calculated between each measurement of the echogenicity of the masseter and temporalis muscles of both sides. A high ICC is set on 0.90.

**Ethical aspect**

The participants were informed about the study by an information letter (English appendix III, Dutch appendix V). They were as well asked to sign the informed consent (English appendix IV, Dutch appendix VI) before they started the experiment. The participants were informed they could leave the study at any time and they did not have to give any information about why they want to quit the study. Every participant got a number and no names were linked to this number, thus the data was stored anonymously. If the participants were interested they were offered the opportunity to receive an email with the results of the study. The data analysis was done in SPSS and Excel. The data will be stored for 15 years at Fontys University of applied Science Eindhoven and the code for the data will be hold at Fontys Eindhoven.
3. Results

In total 102 participants participated. There were 53 women (52%) and 49 man (48%), taken together, with a mean age of 22.5±1.60 years. Mean BMI for women was 23.7±3.20 and for men 23.5±2.35. The intrarater reliability of the measurements was high with an Intra Class Correlations (ICC's) of 0.933 or higher. Two different devices were used. 43 participants were measured with device 1 and 53 participants with device 2. Data of 6 participants was missing. Characteristics of the participants measured with device 1 are shown in table 1. Device 2 is not used for reference values.

Table 1. Characteristics of study participants device 1 (N=43) (mean ± SD(range))

<table>
<thead>
<tr>
<th>Number of participants</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>Preferred chewing side</th>
<th>Non-preferred chewing side</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Masseter Echogenicity</td>
<td>Echogenicity Temporals</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>22.2±1.32</td>
<td>24.1±3.79</td>
<td>81.3±12.70 (58.3 to 107.3)</td>
<td>81.9±15.50 (53.3 to 108.6)</td>
<td>83.5±14.42 (44.7 to 107.6)</td>
</tr>
<tr>
<td></td>
<td>(20 to 24)</td>
<td>(19.0 to 32.0)</td>
<td></td>
<td></td>
<td>81.4±17.42 (35.7 to 110.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>82.4±11.68 (51.5 to 105.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>81.7±14.21 (44.5 to 104.0)</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>22.5±1.66</td>
<td>22.8±2.07</td>
<td>67.5±12.98 (46.6 to 92.0)</td>
<td>77.2±14.89 (55.1 to 105.9)</td>
<td>74.9±13.55 (56.1 to 97.1)</td>
</tr>
<tr>
<td></td>
<td>(20 to 25)</td>
<td>(20.1 to 27.0)</td>
<td></td>
<td></td>
<td>71.8±15.03 (43.9 to 93.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>70.4±10.97 (53.9 to 94.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>74.7±10.97 (56.8 to 97.0)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>22.3±2.12</td>
<td>23.6±3.26</td>
<td>75.9±14.38 (46.6 to 107.3)</td>
<td>80.2±15.23 (53.3 to 108.6)</td>
<td>80.1±14.55 (44.7 to 107.6)</td>
</tr>
<tr>
<td></td>
<td>(20 to 25)</td>
<td>(19.0 to 32.0)</td>
<td></td>
<td></td>
<td>77.6±17.00 (35.7 to 110.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>77.7±12.74 (51.5 to 105.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>78.9±13.33 (44.5 to 104.0)</td>
</tr>
</tbody>
</table>

SD= Standard Deviation, kg=kilograms, m=meters, BMI= Body Mass Index. * = Significant difference (p-value=0.001) between men and women, **= Significant difference (p-value= 0.048) between masseter muscle preferred chewing side and non-preferred chewing side.

There was a significant difference found with a paired t-test between the echogenicity of the preferred (M= 75.9, SD=14.38) and non-preferred chewing side (M=80.1, SD=14.55) for the Masseter muscle with a p-value of 0.048, taken men and women together (N=43). No significant difference was found in the Temporalis muscle between the preferred (M=80.2, SD=14.55) and non-preferred (M=77.6, SD=12.74) chewing side with a p-value of 0.359, taking men and women together.

Significant differences were found between the echogenicity of the Masseter muscle between men and women. Women have a significant higher echogenicity on the preferred chewing side (M=81.3, SD=12.70, men: M=67.5, SD=12.98) for the Masseter muscle (p=0.001) then men. For the non-preferred chewing side there was no significant difference found between men and women, but a trend was found with p-value of 0.058. The average of the echogenicity of the Masseter muscle was significant different (women: M=82.4, SD=11.68, men: M=70.4, SD=10.98) (p=0.002) between the two sexes.
For the Temporalis muscle there were no significant differences found between men and women for neither preferred (p-value=0.370), non-preferred (p-value=0.070) nor average echogenicity (p-value=0.094). Instead a trend was found for non-preferred chewing side (p-value=0.070) and for average echogenicity of the Temporalis muscle (p-value=0.094).

In table 3 correlations are shown between main variables of this study (BMI, echogenicity of preferred and non-preferred chewing side) within all the participants (N=102). A moderate correlation coefficient was set on a coefficient between 0.60 and 0.80. All correlation coefficients are moderate or weak. There was one significant moderate correlation, which is between the echogenicity of the preferred chewing side and non-preferred chewing side of the Masseter muscle. This is visually represented in figure 7. A significant weak correlation was found between preferred and non-preferred chewing side of the Temporalis muscle, visually represented in figure 8. A significant weak correlation coefficient is found between the both preferred chewing sides from the Masseter muscle and Temporalis muscle. Visually represented in figure 9.

Table 3. Correlations between main variables (correlation coefficient (p-value))

<table>
<thead>
<tr>
<th>Echogenicity preferred chewing side</th>
<th>Echogenicity non-preferred chewing side</th>
</tr>
</thead>
<tbody>
<tr>
<td>Masseter</td>
<td>0.122 (p=0.220)</td>
</tr>
<tr>
<td>Temporalis</td>
<td>0.028 (p=0.781)</td>
</tr>
<tr>
<td>Masseter</td>
<td>0.053 (p=0.600)</td>
</tr>
<tr>
<td>Temporalis</td>
<td>0.553 (p&lt;0.001)</td>
</tr>
<tr>
<td>Masseter</td>
<td>0.116 (p=0.245)</td>
</tr>
<tr>
<td>Temporalis</td>
<td>0.748 (p&lt;0.001)</td>
</tr>
<tr>
<td>Masseter</td>
<td>0.531 (p&lt;0.001)</td>
</tr>
<tr>
<td>Temporalis</td>
<td>0.540 (p&lt;0.001)</td>
</tr>
<tr>
<td>Masseter</td>
<td>0.595 (p&lt;0.001)</td>
</tr>
<tr>
<td>Temporalis</td>
<td>0.549 (p&lt;0.001)</td>
</tr>
</tbody>
</table>

BMI= Body Mass Index, p= p-value, BMI were not normally distributed so when involved Spearman’s rho are used. All the other correlations are Pearson’s r. (N=43)

![Figure 7](image) – Correlation between echogenicity of the preferred and non-preferred chewing side from the m. Masseter
Figure 8 – Correlation between echogenicity of the preferred and non-preferred chewing side from the M. Temporalis

Figure 9 – Correlation between echogenicity of the preferred chewing side from the m. Masseter and from the m. Temporalis
4. Discussion

The main aim of this study was to assess the echogenicity of the Masseter muscle and the Temporalis muscle in students within the age of 20 till 26 years. Secondly the influence of BMI, sex and preferred chewing side was determined. The mean echogenicity of the Masseter muscle measured with device 1 was 77.9±12.29 and the mean echogenicity of the Temporalis muscle was 80.7±13.71 (N=43). No studies have been done about the echogenicity of the Masseter and Temporalis muscles, therefore there are no reference values. Echogenicity of the Quadriceps Femoris muscles was studied before. Values for the echogenicity in a young population (M=24.2, SD=3.7) were: Rectus Femoris muscle 101.9±14.0, Intermedius muscle 77.7±20.1, Vastus Lateralis muscle 94.3±17.8 and Vastus Medialis muscle 96.2±17.8 (15).

Other studies found an increase in echogenicity in women compared to men, among others, Quadriceps Femoris muscles, Biceps Brachii muscles, Sternocleidomastoideus muscle and Tibialis Anterior muscles (17,20). In the current study a significant higher (p-value = 0.002) echogenicity in women compared to men for the Masseter muscle was found. For the Masseter muscle women showed an echogenicity of 82.4± 11.68 and men 70.4 ± 10.97. Additionally, the echogenicity of the Masseter muscle on the preferred chewing side was significant higher for women compared to men(p-value =0.002). The mean echogenicity on the preferred chewing side for women was 81.3 ± 12.70 and for men 81.9 ±15.50.

In the analysis there was no significant correlation found between BMI and the echogenicity of both muscles. This result was similar to the findings in the study done by Fukumoto et al. (21),which found no significant correlation between the echogenicity of Quadriceps Femoris muscles and BMI in middle-aged and elderly women. Contrary to this Satiroglu et al. (22) found a significant correlation between Masseter muscle thickness and BMI in young adults. Secondly, Caresio et al. (17) found a positive correlation between BMI and the echogenicity of the Vastus Lateralis, Tibialis Anterior and Medial Gastrocnemius muscles. This means that there is no consensus in literature yet about the influence of BMI on the echogenicity of muscles. Further research with a greater amount of participants in a wide range of BMI should be done to determine the relationship between echogenicity and BMI.

In this study 80.4% of the participants (N=102) preferred to chew on the right side, which is in line with other studies (23–25). There was a significant difference in echogenicity found between preferred and non-preferred chewing side for the Masseter muscle (N=43, p-value=0.048). Other studies by Volk et al. (26) and Caresio et al. (17) found no significant difference between sides which were preferred. Volk et al.(27) found no significant differences in muscle thickness, muscle area and contractility of the facial muscle between preferred chewing side and non-preferred chewing side. Caresio et al. (17) found no significant differences in echogenicity between the dominant side and non-dominant side of the Biceps Brachii and four lower extremity muscles. The difference found in this study was slightly significant for
the Masseter muscle (p-value = 0.048), but for the Temporalis muscle no differences were found. Taken the complete participants population (N=102), there were no significant differences found between preferred and non-preferred chewing side for both Masseter and Temporalis muscles.

The results of this study showed a significant moderate correlation (r = 0.749; p-value < 0.001) between preferred and non-preferred chewing side of the Masseter muscle. Suggested could be that a high echogenicity of the preferred chewing side is combined with a high echogenicity of the non-preferred chewing side. A significant weak correlation (r = 0.595; p-value < 0.001) between preferred and non-preferred chewing side of the Temporalis muscle was found. Subsequently, the relation between the echogenicity of the preferred and non-preferred chewing side of the Masseter muscle was stronger than the relation between the echogenicity of the preferred and non-preferred chewing side of the Temporalis muscle. There was a significant weak correlation (r = 0.553; p-value < 0.001) found between the echogenicity of the preferred chewing side of the Masseter muscle and preferred chewing side of the Temporalis muscle. The function of the Temporalis muscle is closing the jaw and stabilising the TMJ (27). But it is not known what the effect is on the echogenicity of the muscle.

**Strengths and weaknesses**

A weaker point of this study was that both researchers were, before the study and workshops, not familiar with the use of the MSU device. Although they had a couple of practising hours and workshops, they were more experienced at the end of the study than in the beginning.

A standardised protocol was used to limit bias between the researchers. Due to difference in anatomical structures, especially in the Temporalis muscle (28), the transducer was placed in different angels and/or positions to get clear images. The borders of the muscles were not always clear lines, due to the pervious mentioned fact, what made it more difficult to select the borders of the muscles. This was more of an issue in the Temporalis muscle than in the Masseter muscle. Both researchers were right-handed, but scanned the muscles on the right side with their left hand to be able to operate the MSU device. This could result in less accurate imaging.

Unless the standardised protocols there were some more weaknesses. The experiment took place in different locations with different lighting. Lights were always turned off but the light coming into the room was not always controllable. This could affect the images taken with the MSU device. As a result, an image could contain more white and this could cause a higher echogenicity.

A second weakness is that one device (device 2) changed gain settings automatically to 35% in between two participants. Manually the gain settings were changed back to 65%. During the analysis of the data, the findings showed a difference in echogenicity compared to the other device. Mean values were higher and after analysing the images one could conclude that there was more grey on these images compared
to the images taken by device 1. A study by Arts et al. (20) found a significant difference between echogenicity values between different ultrasound devices. They concluded that “normative echo intensity values can only be used with the same ultrasound device and settings; in other cases, new values must be established.” (p.40) (20). This was one of the reasons this study used two devices which were completely the same. But the findings showed different values and further research could analyse this. Reimers et al. (14) found that the echogenicity of muscles change when the gain of a ultrasound device is different. Correlational analysis within a subject was done with data of both devices, because when the Masseter muscle was measured the Temporalis muscle was measured with the same device and settings. Therefore, the relation between both muscle is not much affected.

Morphology was not taken into consideration. A study by Kiliardis et al. (29) found a significant relation between Masseter muscle thickness and facial morphology in women. Women with a longer face had thinner Masseter muscle thickness. Satiroglu et al. (22) analysed this as well and found a comparable outcome. The outcome was a significant relation between Masseter muscle thickness and facial morphology in both men and women. When a muscle is smaller this could affect the echogenicity of the masticatory muscles as well. Thus, for next studies it can be recommended to take this into consideration as well.

A strength of this study was the fact that the intrarater reliability ICC-values were 0.933 or higher. In previous studies the interrater reliability was found to be moderate to weak with values from 0.539 (p-value< 0.001) till 0.616 (p-value < 0.001) (30) for measuring the muscle’s thickness.

No studies have been done about the echogenicity of the masticatory muscle. However, echogenicity could tell something about the quality of a muscle and it is known that good masticatory function declines the risks for stroke and developing dementia. Therefore, this study was a good start for analysing the echogenicity of masticatory muscles starting with a young and healthy population.

**Recommendations for further research**

This study had participants in the age range from 20 till 26 years old. This study can be done with different age ranges to get normative values and determine if there are echogenicity differences when increasing the age. A few studies which have been done about the echogenicity of muscles show an increase in the echogenicity (15,16). Besides this study no study has been published about the echogenicity of the masticatory muscles. Subsequently, to give information about the change in echogenicity with age new studies need to be done.

Recommendation for further research is to study the difference in echogenicity of the masticatory muscles between a population with TMD’s and without TMD’s to find any changes in muscle quality due to TMD’s. When more results are found about the change in echogenicity and muscle thickness of the
masticatory muscles physiotherapists can use the MSU device to assess the function of the masticatory muscles when TMD is suspected.

This study used different devices which could influence the outcome of the study. A study by Arts et al. (20) found that normative values for the echogenicity could only be used with the same device and settings. Thus, for further research could investigate the ICC between different ultrasound devices. One should make sure the settings are exactly the same between the devices to get normative values which can be used.

Facial morphology is known to be have an influence on Masseter muscle thickness (22,29). For further research it is recommended to take this into account. As well as the relationship between Temporalis and Masseter muscle could be investigated. One could investigate if the muscles both have the same function or one is the prime mover and the other is a secondary mover.

In this study chewing side preference was self-reported by the participant. A great portion of the participants did not know what side they preferred to chew. The researchers gave them the opportunity to experience it after the images were taken. Yamasaki et al. (18) found an objective assessment to analyse the actual chewing side with electromyographic (EMG) activity for the Masseter muscle. Therefore, this could be used to analyse the actual chewing side preference objectively, which can be taken into consideration when analysing preferred chewing side echogenicity compared to non-preferred chewing side echogenicity.

**Practical relevance**

MSU is an accessible and low cost way to assess the masticatory muscle in primary care takers settings. Echogenicity gives information about the muscle’s quality. For this studied age group it is not relevant to use echogenicity for assessing the echogenicity in healthy people. It could be used in other age groups, but the relevance of this should be determined in further studies. The change in echogenicity could be used to assess the masticatory muscles function in elderly people or in people which are suspected for TMD’s. Only the impact of aging or TMD’s on the echogenicity of the masticatory muscles should be investigated.
5. Conclusion

In conclusion, the echogenicity of the masticatory muscles is higher in women compared to men. Preferred chewing side tends to have a weak influence on the echogenicity of the masticatory muscles. In the Masseter muscle this seems to have more influence compared to the Temporalis muscle. There was no relation found between the echogenicity of the masticatory muscles and BMI. Despite the limitations of this study the reference values found with device 1 can be used for the age group of 20 until 25 years. Reference values for the Masseter muscle were for women 82.4 ± 11.68 and for men 70.4 ± 10.97 and for the Temporalis muscle for women and men respectively 81.7 ± 14.21 and 74.7 ± 10.97. Further research should be done for different age groups and to find changes in echogenicity due to TMD’s.
6. Literature


Appendix I

Flow chart data collection procedure

Two participants enter into the room.

- Give information about the research and what the participant can expect.
- Give the information letter and informed consent.
- Any questions?

- Give questionnaire about chewing and open mouth habits.

STATION B
> MSU of the temporalis and masseter m. thickness
> 3 times on both sides for masseter in relaxed state
> 3 times on both sides for masseter contracted
> 3 times on both sides for temporalis in relaxed state
> 3 times on both sides for temporalis contracted.

STATION A
> MSU of the temporalis and masseter m. thickness
> 3 times on both sides for masseter in relaxed state
> 3 times on both sides for masseter contracted
> 3 times on both sides for temporalis in relaxed state
> 3 times on both sides for temporalis contracted.

> Measure weight and height without shoes

> Measure weight with empty pockets and no belt.

> Calculation of the BMI: weight in kilograms / (height in m * height in m)

Finish
## Protocol for data collection

<table>
<thead>
<tr>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Introduction</strong></td>
</tr>
<tr>
<td>- Explanation about thesis subject.</td>
</tr>
<tr>
<td>- Information about the experiments, the measurements and why there is a collection of these data.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Information Letter</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>- The participant will be asked if she/he has read the information letter and if she/he has any questions or comments related to the research.</td>
</tr>
<tr>
<td>- (English: Appendix III, Dutch: Appendix V)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Consent agreement</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>- The consent agreement must be read and signed by the participant</td>
</tr>
<tr>
<td>- Then the experiment can start.</td>
</tr>
<tr>
<td>- (Englisch: Appendix IV, Dutch: Appendix VI)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Questionnaire</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>- The participant will be asked for his/her age, seks, preferred chewing side and open mouth habits.</td>
</tr>
<tr>
<td>- (Appendix IX)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>BMI</strong></th>
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<tbody>
<tr>
<td>- The participant is asked to take of their shoes, belt and empty their pockets.</td>
</tr>
<tr>
<td>- The height is measured with a calibrated ruler, which is fixed to the wall and the measure will be in m.</td>
</tr>
<tr>
<td>- The weight is measured with a weighing scale, which will be the same for all participant and the measure will be in kg.</td>
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</tbody>
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<table>
<thead>
<tr>
<th><strong>MSU</strong></th>
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<tbody>
<tr>
<td>- Images of Temporalsis and Masseter muscle will be taken and saved</td>
</tr>
<tr>
<td>- See protocol measurements.</td>
</tr>
<tr>
<td>- (Appendix VII)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Order of Measurements</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Two patients per half an hour will enter into the room</td>
</tr>
<tr>
<td>- Introduction and information letter will be given</td>
</tr>
<tr>
<td>- Signature of consent agreement by the participant and the assessors.</td>
</tr>
<tr>
<td>- Fill in questionnaire about age, seks, preferred chewing side and open mouth habits.</td>
</tr>
<tr>
<td>- BMI station</td>
</tr>
<tr>
<td>- MSU station</td>
</tr>
<tr>
<td>- Goals: - To assess two participant per half an hour.</td>
</tr>
<tr>
<td>- To assess around 100 persons.</td>
</tr>
<tr>
<td>- (Appendix I)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Image Analyse</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Images are saved on flash drive.</td>
</tr>
<tr>
<td>- Images are analysed with ImageJ.</td>
</tr>
<tr>
<td>- (Appendix VIII)</td>
</tr>
</tbody>
</table>
Appendix III

English Information letter

Dear Sir and madam,

We would like to invite you to be part of our Physiotherapy research project at Fontys Paramedische Hogeschool in Eindhoven. Please, read attentively this file to know, if you can be part of our project.

Topic of our research:
Physiotherapists take care of people with body disorders like muscle, tendon or joint pain. While, we are getting older, our body becomes weaker and can lead to many disorders. One of them can be jaw disorder. May be, you have noticed that elderly people eats smaller portions of food compared to healthy adults. This state can be due to chewing difficulties related to chewing muscles weakness. That's why, we are making a project to find relationships between body weight, height and muscles thickness of the jaw by using ultrasound (echoes). Furthermore, we are going to observe the quality of your muscles by using echoes.

Conditions for participation in the research
- You’re between 20 to 25 years old,
- You’re able to understand English language,
- You’re a healthy woman or man,
- You haven’t had any jaw disorders for the last 6 months.
- You’ve never had any jaw surgery,
- You don’t miss cognitive skills, which impede you to participate and understand the experiment,
- You didn’t have any broken teeth or tooth infection last month.
- You didn’t remove your wisdom teeth last month.

Measurements:
3 measurements are made:
- 24 images by ultrasound (echoes).
- BMI (measure body weight and height).
- Questionnaire.

Introduction: You will enter into the room. First of all, we will give you a short introduction to explain how the experiment will be carry on. Then you will be asked to fill in a questionnaire. We will always be present to respond to your question.

Echo: A little bit of gel will be spread on a transducer in order to observe your chewing muscles via the ultrasound. You will only feel a cold gel on your cheek when the transducer is placed on your jaw in order to take images of your chewing muscles. On each side there will be two muscles scanned. And 6 images will be made by muscle. Twelve pictures will be taken when your muscles are in relaxed state. Then 12 others pictures will be done when you are clenching your teeth. In total there will be 24 images made. These images are taken in order to collect muscles thickness and muscles quality.

BMI: We will measure your weight and height. For measuring your height your hair will be compressed. For determining your weight and height we will ask you to take off your shoes, to empty your pockets and to take of your belt.

All measuring equipment will be cleaned with alcohol between measurements. All the information will be secret and anonymously.
What will happen to your data?
All data is anonymous and will be used only for medical purposes. However, we would like to save the data after the research to use them for other researches. In any case your name will never be written on our research project. You are free to get your results, just let us know if you want them and give us your email address.

What do you have to do?
We invite you to join us at Fontys School building in Eindhoven.

Any negative points?
We will make sure that the measurements are free from pain or discomfort.
It will take around 30 minutes to measure everything.
If you feel any embarrassment during our assessment you are free to leave whenever you want.
Participation is completely voluntary and if you can’t participate, we will invite you to share it to your friends or family members.
In any case, thank you for your attention.

If you have any others questions, please let us know.

You can contact us via ricourguim@hotmail.fr or hanneke.vandersteen@student.fontys.nl

Thanks in advance for your time and your consideration,

Kind regards,

Guillemette Ricour
Hanneke Van der Steen
Daniel Leeuwen
Appendix IV

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 question that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Name of participant

Signature of participant

Date __________________________ Day/month/year of consent

Filled in by the researcher:
I have explained the purpose of this research and what can be expected during the study. I have given the participant all information about the study. And the participant is allowed to quit the study at any time they want.

Signature of researcher I

Date __________________________

Signature of researcher II

Date __________________________
Appendix V

Nederlandse Informatie brief

Beste meneer en mevrouw,

Wij willen jou uitnodigen om deel te nemen aan ons fysiotherapie onderzoek op Fontys Paramedisch Hogeschool in Eindhoven. Wil je deze brief met aandacht doorlezen en kijk of jij geschikt bent om deel te nemen aan ons onderzoek.

Onderwerp van ons onderzoek:
Fysiotherapeuten zorgen voor mensen met lichamelijke klachten, zoals spier-, pees- of gewrichtspijn. Terwijl we ouder worden, wordt ons lichaam zwakker en dit kan leiden tot veel stoornissen. Een van deze stoornissen kan kaakpijn zijn. Misschien is het je opgevallen dat oudere mensen kleiner porties voedsel eten in vergelijking met jongeren. Dit kan een gevolg zijn van kauwproblemen gerelateerd aan kauwspier zwakte. Daarom zoeken wij in dit project naar een verband tussen lichaamsgewicht, lengte en spierdikte van de kaakspieren, gebruikmakend van ultrasound (echografie), daarbij ook kijkend naar de kwaliteit van de kaakspieren.

Criteria voor de deelnemers aan ons onderzoek:
- Jij bent tussen de 20 en 25 jaar oud
- Jij bent in staat om de Engelse taal te begrijpen
- Jij bent een gezonde man of vrouw
- Jij hebt geen kaakklachten gehad in de laatste 6 maanden
- Jij hebt nooit een kaakoperatie gehad
- Jij beschikt over goede cognitieve functie, wat inhoudt dat je wilsbekwaam bent en het onderzoek begrijpt
- Jij hebt geen gebroken tanden of tandinfecties gehad in de laatste maand
- Jij hebt in de laatste maand geen verstandskiezen laten verwijderen

Metingen:
Er worden 3 metingen gedaan:
- 24 afbeeldingen met ultrasound (echografie)
- BMI (gewicht en lengte)
- Vragenlijst

Introductie: Na binnenkomst zal er eerst een korte introductie geven worden over hoe het onderzoek wordt uitgevoerd en wat er van jou verwacht wordt. Daarna zal gevraagd worden om een korte vragenlijst in te vullen, we zullen altijd aanwezig zijn om eventueel vragen te beantwoorden.

Echo: een klein beetje gel zal op de transducer worden gedaan om ervoor te zorgen dat de kauwspieren goed zichtbaar zijn op de echo. Dit kan een beetje koud aanvoelen als de transducer op de kaak wordt geplaatst. Aan elke kant worden twee spieren bekeken en van elke spier worden 6 afbeeldingen gemaakt, er worden twaalf afbeeldingen gemaakt van de ontspannen spieren en 12 van de aangespannen spieren. De aanspanning van de spieren wordt bereikt door de kaken op elkaar te bijten. De afbeeldingen worden gemaakt om de spierdikte en de spierkwaliteit te beoordelen.
BMI: Wij zullen jouw gewicht en lengte meten. Voor de lengtemeting vragen we je om de schoenen uit te doen, misschien worden ook je haren platgeduwd bij het meten. Ook bij het wegen vragen we je je schoenen uit te doen en je zakken leeg te maken en (wanneer gedragen) je riem af te doen.

Tussen de metingen door zullen alle meetinstrumenten schoongemaakt worden. Alle informatie zal zorgvuldig en anoniem worden opgeslagen.

Wat gebeurt er met de data?
Alle data zal anoniem worden opgeslagen en alleen voor medische doeleinden worden gebruikt. Alleen willen we de data na het onderzoek bewaren, zodat andere onderzoekers deze data kunnen gebruiken in toekomstig onderzoek. In geen geval zal jouw naam worden gekoppeld aan de data. Jij kunt de resultaten van het onderzoek ontvangen, dit hoeft je alleen maar te laten weten door jouw e-mailadres achter te laten.

Wat moet jij doen?
We zullen jou uitnodigen om ons te ontmoeten in het Fontys gebouw in Eindhoven. Om je te bedanken zullen we je een kleine attentie geven.

Enkele mogelijk negatieve punten van het onderzoek:
We zullen ervoor zorgen dat de metingen pijnloos en comfortabel zullen verlopen. Het zal ongeveer 30 minuten duren om alle metingen uit te voeren. Wanneer je pijn of schaamte voelt tijdens het onderzoek, dan ben je altijd vrij om het onderzoek op elk moment te stoppen. Deelnemen is volledig vrijwillig en wanneer je niet kunt deelnemen, hopen wij dat je deze informatie deelt met je vrienden of familie, zodat zij de mogelijkheid hebben om wel deel te nemen.

Als je nog vragen hebt, laat dit alsjeblieft weten. Je kunt contact op nemen met ons via ricourguim@hotmail.fr of hanneke.vandersteen@student.fontys.nl

In elk geval bedankt voor je aandacht.

Met vriendelijke groet,

Guillemette Ricour
Hanneke Van der Steen
Daniel Leeuwen
Appendix VI

Informed consent (Dutch)

Bewijs van overeenkomst:
Ik heb de voorafgaande informatie gelezen, of het is mij voorgelezen. Ik heb de mogelijkheid gehad om vragen te stellen en elke vraag is naar mijn tevredenheid beantwoord. Ik geef vrijwillig toestemming om deel te nemen aan dit onderzoek.

Naam van deelnemer _______________________________________

Handtekening van deelnemer_________________________________

Datum __________________________ Dag/maand/jaar

Invullen door onderzoeker:

Ik heb uitgelegd wat het doel van dit onderzoek is en wat er verwacht kan worden tijdens het onderzoek. Ik heb de deelnemer alle informatie over het onderzoek gegeven. En de deelnemer is toegestaan om het onderzoek op elk moment te stoppen.

Handtekening van onderzoeker I _______________________________

Datum ______________________________________________________

Handtekening van onderzoeker II _______________________________

Datum ______________________________________________________
## Appendix VII

### Protocol measurements ESAOTE My Lab One with 13.0MHz transducer;

<table>
<thead>
<tr>
<th>Step Plan</th>
<th>Explanation</th>
</tr>
</thead>
</table>
| **Start using ultrasound** | - Connect the transducer to the display,  
- Turn the ultrasound system on by pressing ON/OFF button  
- It takes around 2 minutes to warm up. |
| **Instructions for the participant** | - A brief introduction about ultrasound and what they can expect about the measurement,  
- Information about relaxation: they are not allowed to laugh, speak, move and smile during the assessment.  
- Information on the biting intensity during the contracted measurements: they must give their best and bite as strong as they can, according to their own limits.  
- Information about the gel which will be on the transducer and a bit on their cheek. |
| **Measurements masseter muscle** | - Place gel on the transducer  
- Place the transducer transverse to the masseter m. of the participant.  
- Make sure the settings are right:  
  - Gain: 65% (between 62% to 68%)  
  - Frequency 13 MHz  
  - Depth 3 cm  
- Place the transducer with the marker pointing towards the left.  
- Parallel to the jaw line.  
- Aligned with the earlobe.  
- Repeat the measurements on both sides for relaxed and contracted states of the muscle  
- There will be 12 images of the masseter muscle |

![Figure 1: observation of the masseter muscle by MSU](image)

- Place gel on the transducer  
- Place the transducer with the marker pointing towards the left,
### Measurements temporalis muscle

- Make sure the settings are right:
  - Gain: 65% (between 62% to 68%)
  - Frequency 13 MHz
  - Depth 4 cm
- On the line between the orbit and the top of the ear, 1 cm upper to the zygomatic arch.
- Place the transducer transverse to the temporalis muscle of the participant.
- Repeat the measurements on both sides for relaxed and contracted state of the muscle.
- There will be 12 images of the Temporalis muscle.

![Observation of the temporalis muscle by MSU](image)

### Results

- Pressing on “FREEZE”.
- Placed 2 points on the echo and draw a line to get the muscle thickness (see appendix V).
- The images will be saved.
- Each participant has a number, which is the first number of the filename (ex: 001).
- The participant has three relaxed and three contracted images.
- Masseter muscle measurements on each side so 12 echoes.
- The tester needs 12 echoes as well for the temporalis muscle.
- In total there is 24 measurements per participant.

### Cleaning

- The transducer is cleaned after each participant.
## Appendix VIII

### Protocol measurements echogenicity with ImageJ

<table>
<thead>
<tr>
<th>Steps</th>
<th>explanation</th>
</tr>
</thead>
</table>
| **Start working in ImageJ** | • Open the program ImageJ  
• Open the first image taken with MSU |
| **Adjust image** | • First scale the image to make sure that when analysing one respects the original size. |
| **Selection ROI** | • Select: “polygon selection”  
• Draw the lines from the outside of the muscle. The ROI should be as big and width as possible. Not the sides of the images; not the fascia of the outer part of the muscle; fascia in the muscle do include!! |
| **Determining echogenicity** | • Go to analyse  
• Go to histogram  
• The number underneath mean is the echogenicity of the muscle, values also to be used are Standard deviation and count |
| **Repeat measurements for the other 2 images of the relaxed muscle** | • Repeat the ROI selection and the calculation for echogenicity. |
| **Repeat measurement for the other side** | • Repeat steps |

---

**ROI selection for the Masseter muscle 1**

**ROI selection for the Temporalis muscle 1**
Appendix X

Geheimhoudingsverklaring

Naam: Hanneke van der Steen
Studentnr°: 2207020

Titel:
Assessment of the echogenicity of the Masseter and Temporalis muscles

Inhoud (omschrijving):
Scriptie over de referentiewaarden van de echogeniciteit van de Masseter en Temporalis spieren. En daarnaast het beoordelen van de invloed van BMI, geslacht en voorkeurskauwzijde op de echogeniciteit van de Masseter en Temporalis spieren.

1. Fontys Paramedische Hogeschool te Eindhoven verbindt zich door ondertekening van deze verklaring, informatie met betrekking tot de verstrekte gegevens en uit onderzoek verkregen resultaten waarvan in het kader van bovengenoemd project praktijkgericht onderzoek kennis wordt genomen en waarvan bekend is of redelijkerwijs begrepen kan worden dat dit als geheim of vertrouwelijk wordt beschouwd, strikt geheim te houden.

2. Tevens geldt deze geheimhoudingsverplichting voor de werknemers van Fontys Paramedische Hogeschool, evenals voor anderen die op enigerlei wijze uit hoofde van hun functie toegang hebben of kennis nemen van bedoelde informatie.


Student: Begeleider:

Naam: Hanneke van der Steen Naam: Daniel van Leeuwen

____________________________ ____________________________
(handtekening) (handtekening)
Datum: 2 / 6 / 2016 Datum: __ / __ / ______

Coördinator: voor ontvangst

Naam: ______________________________

____________________________
(handtekening)
Appendix XI

Overeenkomst overdracht rechten

OVEREENKOMST

houdende overdracht van rechten en de plicht tot
overdracht/retournering van data, software en andere middelen

Ondergetekenden:
1. de heer/mevrouw Adriana Johanna van der Steen woonachtig te 5291AB, Gemonde aan de Sint Lambertusweg 103b hierna aan te duiden als “Student”

en

2. Stichting Fontys h.o.d.n. Fontys Hogescholen, Rachelsmolen 1, 5612 MA Eindhoven, hierna “Fontys”

CONSIDERANS

A. Student studeert aan de Fontys Paramedische Hogeschool te Eindhoven en heeft in het kader van zijn/haar studie, al dan niet tezamen met derden en/of in opdracht van derden, (diverse) activiteiten verricht, of zal deze nog verrichten, in het kader van onderzoeken die onder supervisie staan van het lectoraat van Fontys Paramedische Hogeschool. Voornoemde activiteiten zullen hierna worden aangeduid als “Lectoraat Studieactiviteiten”. Ten tijde van ondertekening van deze verklaring superviseert het lectoraat van Fontys Paramedische Hogeschool in ieder geval de onderzoeken die zijn opgesomd in bijlage 1, maar deze opsomming is niet uitputtend en kan in de toekomst veranderen.

B. Het is voor Fontys Paramedische Hogeschool van essentieel belang dat (uitwerkingen van) de Lectoraat Studieactiviteiten ongehinderd en zonder enige beperking verder kunnen worden ontwikkeld en toegepast door Fontys Paramedische Hogeschool en/of voor onderwijs van andere studenten kunnen worden gebruikt. Fontys wil in ieder geval – maar niet uitsluitend – (uitwerkingen van) de Lectoraat Studieactiviteiten (i) kunnen delen met en/of over te dragen aan derden, (ii) op eigen naam kunnen publiceren, waarbij Student mogelijk als co-auteur kan worden vernoemd mits dit gezien de omstandigheden redelijk is, (iii) kunnen gebruiken als basis voor nieuwe onderzoeksprojecten.

C. Als op (uitwerkingen van) Lectoraat Studieactiviteiten intellectuele eigendomsrechten (komen te) rusten en/of daaraan verwante aanspraken van Student, wensen partijen – het onder (B) genoemde in aanmerking nemend – dat Fontys Paramedische Hogeschool de enige rechthebbende ten aanzien van deze rechten en aanspraken is. Student wenst dan ook al zijn/haar huidige en
toekomstige intellectuele eigendomsrechten alsook daaraan verwante aanspraken met betrekking
tot (uitwerkingen van) de Lectoraat Studieactiviteiten aan Fontys over te dragen, onder de hierna te
noemen voorwaarden;

D. Student wenst voorts de verplichting op zich te nemen – wederom het onder (B) genoemde in
aanmerking nemend – om alle door hem/haar in het kader van (uitwerkingen van) de Lectoraat
Studieactiviteiten door hem/haar verzamelde data aan Fontys over te dragen en geen kopieën
daarvan te bewaren, en eveneens alle in het kader van (uitwerkingen van) de Lectoraat
Studieactiviteiten aan hem/haar door Fontys verstrekte eerder verzamelde data, software en/of
andere middelen, zoals meet- en testapparatuur, aan Fontys te retourneren zonder kopieën daarvan
tebewaren, dit alles onder de hierna te noemen voorwaarden.

KOMEN OVEREEN ALS VOLGT

1. Overdracht intellectuele eigendomsrechten

1.1 Student draagt hierbij over aan Fontys Paramedische Hogeschool al zijn/haar huidige en
toekomstige intellectuele eigendomsrechten en aanverwante aanspraken met betrekking tot
(uitwerkingen van) de Lectoraat Studieactiviteiten, voor de volledige duur van die rechten.

1.2 Onder intellectuele eigendomsrechten en/of daaraan verwante aanspraken wordt tenminste –
maar niet uitsluitend – verstaan het auteursrecht, het databankenrecht, het octrooirecht, het
merkenrecht, het handelsnamenrecht, het tekeningen- en modellenrecht, het kwekersrecht,
bescherming van knowhow en bescherming tegen oneerlijke mededinging.

1.3 De in 1.1 omschreven overdracht is onbeperkt. Zodoende omvat de overdracht alle aan de
overgedragen rechten en aanspraken verbonden bevoegdheden, en geldt de overdracht voor alle
landen ter wereld.

1.4 Voor zover enige nationale wetgeving enige nadere medewerking van Student vereist voor de
overdracht genoemd onder 1.1, zal Student deze medewerking op eerste verzoek van Fontys
Paramedische Hogeschool onmiddellijk en zonder enig voorbehoud verlenen.

1.5 Fontys aanvaardt de in 1.1 omschreven overdracht.

2. Afstand van persoonlijkheidsrechten

2.1 Voor zover toegestaan onder artikel 25 Auteurswet, en andere eventueel toepasselijke
nationale wetgeving, doet Student afstand van zijn/haar persoonlijkheidsrechten, waaronder begrepen
– maar niet uitsluitend – het recht op vermelding van de naam van Student en het recht zich te verzetten tegen wijzigen van (uitwerkingen van) de Lectoraat Studieactiviteiten. Indien en voor zover aan Student onder enige nationale wetgeving een beroep toekomt op persoonlijkheidsrechten ondanks het bovenstaande, zal Student zich niet op onredelijke gronden op deze persoonlijkheidsrechten beroepen.

2.2 In afwijking van hetgeen in 2.1 is bepaald kan Fontys Paramedische Hogeschool besluiten de naam van Student wel te vermelden als dit gezien de omvang van zijn/haar bijdrage en werkzaamheden redelijk is.

3. **Vergoeding**

Student gaat ermee akkoord dat hij/zij geen vergoeding voor de in deze verklaring omschreven rechtenoverdracht en -afstand van Fontys zal ontvangen.

4. **Garantie ten aanzien van intellectuele eigendomsrechten**

Student verklaart bevoegd te zijn tot de overdracht en afstand, en verklaart geen licentie(s) tot enigerlei wijze van gebruik van (uitwerkingen van) de Lectoraat Studieactiviteiten aan enige derde(n) te hebben verleend of in de toekomst te zullen verlenen. Student vrijwaart Fontys voor iedere aanspraak van derden in dit kader.

5. **Plicht tot overdracht/retournering van data, software en andere middelen**

5.1 Op het moment dat Student niet langer Lectoraat Studieactiviteiten verricht en/of niet langer student van Fontys is, verplicht Student zich tot overdracht aan Fontys van alle in het kader van (uitwerkingen van) de Lectoraat Studieactiviteiten door hem/haar verzamelde data, in de ruimste zin van het woord, daaronder begrepen – maar niet uitsluitend – onderzoeken en onderzoeksresultaten, tussentijdse notities, documenten, afbeeldingen, tekeningen, modellen, prototypes, specificaties, productiemethoden, procesbeschrijvingen en techniekbeschrijvingen.

5.2 Student garandeert op geen enkele wijze, in welke vorm dan ook, kopieën van de in 5.1 bedoelde data bewaard te hebben.

5.3 Student verplicht zich om aan Fontys te retourneren alle in het kader van de Lectoraat Studieactiviteiten aan hem/haar door Fontys verstrekte data, software en/of andere middelen, en garandeert op geen enkele wijze, in welke vorm dan ook, kopieën van de verstrekte software en/of andere middelen bewaard te hebben.
5.4 Student gaat ermee akkoord dat indien hij in strijd met de verplichtingen en garanties genoemd onder 5.1 tot en met 5.3 handelt en/of gehandeld blijkt te hebben, (a) hij/zij aansprakelijk is voor alle schade die Fontys hierdoor heeft geleden en/of nog zal lijden, en (b) dat dit als fraude kwalificeert en Fontys daaraan passende sancties mag verbinden. De door Fontys op te leggen sancties kunnen onder meer bestaan uit het niet toekennen van studiepunten, het tijdelijk uitsluiten van Ondergetekende van deelname aan examens, maar ook uit het definitief uitschrijven van Ondergetekende als student van Fontys.

6. Afstand

Student doet afstand van het recht op ontbinding van deze overeenkomst.

7. Overig

7.1 Voor zover deze overeenkomst afwijkt van het studentenstatuut geldt dat deze overeenkomst voor gaat.

7.2 Deze overeenkomst wordt beheerst door Nederlands recht. Alle uit deze verklaring voortvloeiende geschillen zullen worden voorgelegd aan de bevoegde rechter te Amsterdam.

Student: Stichting Fontys
h.o.d.n. Fontys Hogescholen
begeleider:

Naam: Adriana Johanna van der Steen

____________________________ (handtekening)   ________________________________ (handtekening)

Datum: 2 / 6 / 2016                      Datum: __ / __ / ______
Plaats: Gemonde                      Plaats: ________________