Masseter and temporalis muscle thickness as assessed by ultrasound – a proof-of-concept study

Amanda Carlsson
Jennifer Mattsson

Supervisor: Per Alstergren, Professor

Master Thesis
Dentistry Programme
February, 2020

Malmö University
Faculty of Odontology
205 06 Malmö
ABSTRACT

Aim: The current study is a proof-of-concept study to investigate the possibility to measure changes in muscle thickness with ultrasound. If this works, the methodology can be used in studies that are currently investigating whether muscle thickness or changes in muscle thickness in use are related to orofacial pain.

Materials and method: Twentytwo healthy research individuals were examined. All of the research individuals answered negatively on the three screening questions for temporomandibular disorders. The ultrasound examination was performed to assess masseter and temporal muscle thickness (thickest part at one standardized location for each muscle) bilaterally as a surrogate measure of total muscle blood flow at rest and during clenching before and after a chewing exercise. The subject would chew three paraffin wax taste-less chewing gums at a frequency of 90 Hz (90 chews per minute) for 5.0 minutes. The subject also answered three questions about muscle pain, fatigue and discomfort before and after chewing.

Result: When comparing the muscle thickness at rest versus clenching, both before and after the chewing exercise, the results were that the masseter muscle is thicker when clenching than at rest. The difference was significant for the masseter but not for the temporal muscle. The results obtained regarding the NRS-scales, which showed an increase in value after the chewing exercise was according to the statistical analysis significant.

Conclusion: This study shows that assessment of muscle thickness with ultrasound is feasible for the masseter muscle since the method is capable of detecting differences between rest and clenching.

Keywords: ultrasound, muscle thickness, TMD, masticatory muscles, proof of concept

SAMMANFATTNING

Syfte: Den aktuella studien är en metodstudie för att undersöka möjligheten att mäta förändringar i muskeltjocklek med ultraljud. Om detta fungerar kan metoden användas i studier som för närvarande undersöker om muskeltjocklek eller förändringar i muskeltjocklek vid användning är relaterade till orofacial smärtor.

Material och metod: Tjugotvå friska forskningsindivider undersöcktes. Alla forskningsindivider svarade negativt på de tre screeningfrågorna för temporomandibulär dysfunktion. Ultraljudsundersökningen utfördes för att utvärdera massetermuskeln och temporalismuskelns tjocklek (tjockaste delen och på en standardiserad plats för varje muskel) bilateralt som ett surrogatmått för det totala muskelblodflödet i vila och under hopbitning före och efter en tuggövning. Individen skulle tugga tre smaklösa paraffintugggummi med en frekvens av 90 Hz (90 tugg per minut) under 5 minuter. Individen svarade också på tre frågor om muskelsmärtor, trötthet och obehag före och efter tuggövningen.


Slutsats: Denna studie visar att bedömning av muskeltjocklek med ultraljud är möjlig för masseter eftersom metoden kan upptäcka skillnader mellan vila och hopbitning.
<table>
<thead>
<tr>
<th>TABLE OF CONTENT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT .............................................................................................................</td>
<td>2</td>
</tr>
<tr>
<td>SAMMANFATTNING ..................................................................................................</td>
<td>2</td>
</tr>
<tr>
<td>INTRODUCTION .........................................................................................................</td>
<td>4</td>
</tr>
<tr>
<td>Pain and temporomandibular disorders .................................................................</td>
<td>4</td>
</tr>
<tr>
<td>Myalgia &amp; Diagnostic Criteria for Temporomandibular Disorders ..........................</td>
<td>5</td>
</tr>
<tr>
<td>Ischemia ...............................................................................................................</td>
<td>5</td>
</tr>
<tr>
<td>Ultrasound ..........................................................................................................</td>
<td>5</td>
</tr>
<tr>
<td>Muscle anatomy and function ...............................................................................</td>
<td>6</td>
</tr>
<tr>
<td>Physiology ..........................................................................................................</td>
<td>8</td>
</tr>
<tr>
<td>Aim .......................................................................................................................</td>
<td>9</td>
</tr>
<tr>
<td>MATERIALS AND METHOD .........................................................................................</td>
<td>9</td>
</tr>
<tr>
<td>Materials .............................................................................................................</td>
<td>9</td>
</tr>
<tr>
<td>Healthy subjects .................................................................................................</td>
<td>9</td>
</tr>
<tr>
<td>Location of the study and collaborators involved in the study ...........................</td>
<td>9</td>
</tr>
<tr>
<td>Sample selection, inclusion criteria and privacy ..............................................</td>
<td>9</td>
</tr>
<tr>
<td>Ultrasound measurements ....................................................................................</td>
<td>10</td>
</tr>
<tr>
<td>Study design .......................................................................................................</td>
<td>10</td>
</tr>
<tr>
<td>Chewing exercise ...............................................................................................</td>
<td>10</td>
</tr>
<tr>
<td>Measurements .....................................................................................................</td>
<td>10</td>
</tr>
<tr>
<td>Outcome variables ..............................................................................................</td>
<td>11</td>
</tr>
<tr>
<td>Statistical methods ............................................................................................</td>
<td>11</td>
</tr>
<tr>
<td>RESULTS ..............................................................................................................</td>
<td>11</td>
</tr>
<tr>
<td>DISCUSSION .........................................................................................................</td>
<td>13</td>
</tr>
<tr>
<td>Methodological considerations ..........................................................................</td>
<td>13</td>
</tr>
<tr>
<td>Result considerations .........................................................................................</td>
<td>15</td>
</tr>
<tr>
<td>Conclusion ..........................................................................................................</td>
<td>16</td>
</tr>
<tr>
<td>REFERENCES .........................................................................................................</td>
<td>17</td>
</tr>
<tr>
<td>APPENDIX 2. INFORMATION TO RESEARCH INDIVIDUAL ...........................................</td>
<td>21</td>
</tr>
<tr>
<td>APPENDIX 3. RESEARCH RECRUITMENT POSTER ....................................................</td>
<td>24</td>
</tr>
</tbody>
</table>
INTRODUCTION

Pain and temporomandibular disorders

There are two types of classified pain, slow and fast. Fast pain is described as stabbing, cutting, electrical etc. It is transmitted by $\delta$-fibers from the periphery to the spinal cord in a speed of 6-30 m/s. Slow pain is described as burning, dull, throbbing etc. This type of pain is transmitted through $c$-fibers and, as the name suggests, in a much slower pace of 0.5-2 m/s. Slow pain can often be associated with tissue deterioration and can lead to extreme suffering. This system is designed to allow a person to quickly react to pain stimulus and therefore remove him- or herself from the object causing the pain and then feel the slow pain a couple of seconds later to be aware of any possible tissue damage. These different signals are transmitted through separate pathways and end up in different laminas of the dorsal horns where they excite second-order neurons and continue towards the thalamus. (1)

One of the most negative and powerful experience a human can have is pain. Pain that lasts a longer period than three months is categorized as chronic pain. Chronic pain in the jaw system can have a severe impact on well-being and decrease the function of the system, (2) such as speaking, eating and expressing emotions. Not being able to do this, because of chronic pain, may therefore have a huge impact on quality of life and daily activities (3,4)

Two studies, performed in the UK, investigated the presence of any type of orofacial pain in 2500 adults and found that between 12% and 19% had some form of pain in the face, mouth or jaw that had lasted for one day or longer in the past month. (5)

TMD pain is the most common chronic orofacial pain disorder and resembles back pain when it comes to intensity, persistence and psychological influence. Although it is uncommon in children before puberty the prevalence in the population spans from 9-15% in women and 3-10% for men. It is up to three times more common in women than in men and prevalence reaches its peak in the age of 35-45 years. TMD pain has quite a low incidence (2-3% per year) and therefore it is suggested that the height of the prevalence is due to its duration rather than onset. (5)

In August of 2015 a study was published where 137 718 individuals were examined for frequent temporomandibular pain by three validated screening questions (3Q). The result was the following; prevalence of frequent temporomandibular pain was 5.2% among women and 1.8% among men (p <0.0001). The prevalence of frequent pain on jaw movement was 2.5% among women and 0.9% among men (p <0.0001). The prevalence of frequent orofacial pain varied between age groups and sex where women in fertile ages showed the highest prevalence, 6-12% of the population. Women had about 2-3 times higher prevalence and the increase in and difference between men and women occurred from 13 years of age. (6) 3Q has excellent sensitivity and specificity to identify cases and controls regarding possible TMD pain diagnoses. The 3Q being:

Q1. Do you have pain in your temple, face, jaw, or jaw joint once a week or more?
Q2. Do you have pain once a week or more when you open your mouth or chew?
Q3. Does your jaw lock or become stuck once a week or more? (7)

In a study published in February 2018, the accuracy of 3Q to find patients with DC/TMD diagnoses was compared to the full diagnostic protocol DC/TMD. They found that if you ask only Q1 or Q2 you are most likely to identify healthy individuals without TMD but not very likely to identify individuals with TMD. If both questions are asked the likelihood to identify
individuals with TMD increases and the likelihood to identify healthy individuals decreases.(7)

Myalgia & Diagnostic Criteria for Temporomandibular Disorders
The Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) has been the most commonly used diagnostic protocol since it was published in 1992 until approximately 2014. (8) In 2014, a new diagnostic protocol was published; the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD).

In DC/TMD, Myalgia is diagnosed with a combination of anamnestic information and data from the clinical examination, including palpation of the temporalis muscle and the masseter muscle. The criteria for diagnosing temporomandibular muscular pain (myalgia) are:
1. In the medical history: pain in jaw, temple, in or in front of the ear in the last month which is affected by jaw movements and function such as chewing or yawning or parafunctions such as tooth clenching or gum chewing.
2. Pain location confirmed by patient and examiner in the temporalis or masseter muscle at the clinical examination AND masticatory muscle pain during palpation of the muscle or maximum assisted or unassisted opening movement.
These criteria have a sensitivity of 0.90 and a specificity of 0.99. (9)

Ischemia
There is a theory that suggests that muscle pain occurs due to muscle ischemia. The theory is that when the oxygen supply to a certain muscle is insufficient to the demand of the muscle, an accumulation of lactic acid begins because the cell metabolism becomes anaerobic and that the lowered pH would cause pain receptors to react. The theory also suggests that other chemical compounds like bradykinin are released upon tissue damage and that these compounds excite nociceptors. (1)

In a study published in 1996 a group of researchers performed experiments on healthy individuals to see if they could find a relation between muscle ischemia and muscle pain. The subjects performed an exercise with the forearm muscles while blood supply was cut-off with a cuff around the arm. Muscle pH was measured with a probe. The other experiment consisted of an injection of an acid phosphate buffer directly beneath the muscle fascia to induce a decrease in pH. In both cases the research individuals experienced pain along with a reduction in pH. (10)

Ultrasound
Ultrasound has the potential to be a method to measure muscle thickness. Ultrasound works by sending soundwaves with a frequency of more than 20 kHz. These frequencies are inaudible to humans. The probe that emits the soundwaves is placed onto the skin with a jelly to avoid air between the skin and the probe. An image is formed when the soundwaves hit tissue and then echoes back into the transducer which converts it into an electrical signal that the ultrasound unit converts into a live image. Ultrasound in the head and neck region is used for example to detect calcifications in salivary glands and ducts, evaluation of the blood flow in the carotid artery and fine-needle biopsies as a guidance. It is also used in general medicine for e.g. prenatal examinations and diagnostics as well as distinguishing different types of arthritis. (11–13)
In 2012 Cartwright et al. published a study were the reliability and validity of ultrasound measurements of muscle thickness was assessed. Their study showed that the validity was very high with a correlation value of 0.985 (P-value <0.001). They also assessed the reliability five ultrasonographers with varying experience in muscle ultrasound that performed measurements of the same sites, which were the biceps brachii and brachialis complex (at the mid-arm) and tibialis anterior (5 cm distal to the tibial plateau). The reliability varied with the level of experience so that the most experienced operators had the highest level of reliability. (14)

Another study was published in 2009 by Thoirs and English in which they researched the intra-examiner reliability and also the impact of which position the patient is in, in this case standing up or lying down. The study showed that the intra-examiner reliability regarding test-retest was high, regardless of the patient's body position. On the other hand, data obtained from the same site in each position were significantly different. The sites in this study included nine sites located on the arms, legs, abdomen and chest. The authors discussed that this could depend on “postural or positional forces acting on muscle shape” or perhaps gravitational forces. (15)

Miyatani et al. published a study in 2002 where the muscle thickness in the femoral quadriceps measured with ultrasound was related to muscle volume in the same muscle as assessed by MRI scans in order to establish whether there would be a relation between the two. The results showed that ultrasound could be an alternative to MRI examinations. (16)

Muscle anatomy and function

The masseter muscle

The masseter muscle originates from the zygomatic arch and extends downward to the lower half on the lateral side of the ramus of the mandible, including the mandibular angle. It is a rectangular shaped muscle and can be divided in two parts, one superficial and one deep part. The directions of the fibers differ in the two parts. The fibers in the superficial part run downward and somewhat posteriorly and in the deep portion the fibers run vertically downward. (17,18)

The masseter muscle is innervated by the massetericus nerve that originates from the mandibularis part of Trigeminal nerve. (17–19) The blood supply of the masseter muscle is provided by a masseteric branch from the maxillary artery. (18,19) It is also supported by the facial artery and the superficial temporal artery through the transverse facial branches. (19)

The main function of the masseter muscle is to elevate the mandible and bring the teeth into contact, which means that it acts mostly as an adductive muscle. It also assists in protrusion of the mandible by stabilizing the condyle against the articular eminence when occlusal force is applied. (17,18)
The temporal muscle

The temporal muscle originates from the lateral surface of the skull and the temporal fossa. The muscle is a large fan-shaped muscle and narrows down and extend between the zygomatic arch and the infratemporal crista and inserts on the coronoid process. According to the function of the muscle and directions of the fibers it can be divided into three different portions, the anterior, the middle and the posterior portion. The muscle fibers in the anterior portion are directed almost vertical. The muscle fibers in the middle portion is slightly directed anteriorly as they continue down towards and inserts at the coronoid process. In the posterior portion the muscle fibers are directed almost horizontally. (17,18,21)

The nervous supply of the temporal muscle is provided by branches from the anterior trunk of the mandibular nerve. (18,19) The blood supply of the temporal muscle is provided by the branches from the temporal artery that originates from the maxillary artery. (19) The anterior region of the muscle is supplied by the anterior deep temporal artery, the middle region is supplied by the posterior deep temporal artery and the posterior region is supplied by the middle temporal artery. (18,19)

The temporal muscle is also a major adductive muscle. However, it consists of three different portions of fibers that all have different directions. The temporal muscle plays an important part in positioning the mandible. If the anterior portion of the muscle in contracted the mandible will move vertically. If the middle portion is contracted the mandible will move upwards and slightly backwards in a retrusive motion. If the posterior portion is contracted the resulting motion of the mandible is debated. (17,18)
Physiology

Muscle physiology
The basic component of the muscle is the motor unit. The motor unit is made up by muscle fibers that are innervated by a motor neuron. The motor neuron is joined with the muscle fibers at what is called the motor end plate. Contraction of the muscle fibers occurs when the neuron is activated and makes the endplate release acetylcholine which causes a depolarization of the fibers. Each motor neuron innervates a different amount of fibers and the lesser the fibers the more precise is the movement. The lateral pterygoid is an example of small amount of fibers innervated by one motor neuron and this makes it possible for this muscle to control the small motions horizontally. In contrast there is the masseter muscle which has a large muscle fiber-to-motor neuron ratio which gives the great force of mastication.

A motor unit can only make the muscle contract but the entire muscle itself can carry out three different functions:

1. A large number of stimulated motor units results in overall shortening of a muscle, e.g. when the masseter elevates the mandible for chewing ability. This is called isotonic contraction and is recognized by shortening under a constant load.
2. A proper amount of motor units contracting against a given force results in an isometric contraction, a contraction of the fibers without shortening of the muscle. For example holding a hard object between the teeth.
3. The third function is the controlled relaxation which means that the stimulation of the motor unit is deliberately terminated and results in a lengthening of a specific muscle or part of muscle. For example the jaw opening motion when eating.

Nerve physiology
The neuron is the basic component of the nerve. It consists of the nerve cell body and the processes called dendrites and axons. The cell bodies in the spinal cord are located in the gray
matter of the central nervous system and the cell bodies outside the central nervous system are bundled together in ganglia. The axon is the main conducting part of the neuron. A nerve fiber is composed by several neurons which are capable of transmitting electrical and chemical signals and thereby get information both to and from the central nervous system. Neurons carrying signals to the central nervous system are called afferent and its counterpart transmitting signals from the central nervous system are called efferent. Nerve signals are sent between neurons through synapses located is the central nervous system.

In every body part there are sensory receptors which are responsible for collecting signals from the periphery to the central nervous system. The receptors responsible for sending signals of discomfort and pain are called nociceptors and the proprioceptors supplies information about positioning and motion of e.g. the mandible and other structures.

The peripheral nerve signals are sent to the central nervous system and then further to the brainstem and the cortex to be interpreted. The interpretation is then sent back via the spinal cord which leads to the tissue from which the signal came from to react in the appropriate way. (18)

Aim
The current study is a proof-of-concept study to investigate whether it is possible to measure muscle thickness and change in muscle thickness with ultrasound. If this works, the methodology can be used in studies that are currently investigating whether muscle thickness or changes in muscle thickness in use are related to orofacial pain. It is currently unknown.

MATERIALS AND METHOD

Materials

Healthy subjects
Twenty-three healthy research individuals were included. Recruitment was conducted among students and staff at the Faculty of Odontology and Faculty of Health and Society at Malmö University through posters and oral information about the study. E-mail addresses were collected and further information was sent to those interested in participating. The subjects were informed about the project and gave their consent before participating.

This project was a proof-of-concept study investigating assessment of masticatory and temporalis muscle thickness with ultrasound. The project was approved by the local Ethical Review Board at the Faculty before recruitment begun (STUD 3.5.3-2019/ 521)

Location of the study and collaborators involved in the study
The study was performed at the Faculty of Odontology, Malmö University, Sweden by the dentistry students Amanda Carlsson and Jennifer Mattsson supervised by Professor Per Alstergren.

Sample selection, inclusion criteria and privacy
The 23 research individuals went through the ultrasound examination after answering the 3Q questionnaire (see Appendix 1) negatively. One of the research individuals was excluded due to insufficient collected data. This leads to a material of 22 research individuals. Out of these 12 were female and 10 were male. The age of the research individuals was 20-57 years old
with a median age of 25 (P$_{25}$ = 23, P$_{75}$ = 40), all healthy with no medication that would affect the measurements.

Each research individual was assigned a code number to be used on forms and in the database. The code list as well as the forms were kept in a locked location with access only by the collaborators in the study. Responsible for the code lists was Per Alstergren. Data analysis was therefore anonymous and performed on a group level.

Inclusion criteria was age 18-75 years and negative answers on the 3Q. Exclusion criteria was limitation in the Swedish language that prevented the subject to answer the research protocol correctly.

**Ultrasound measurements**
The ultrasound used in this study was the LOGIQ™ S7 Expert with a broad-spectrum linear matrix array transducer called ML6-15 with a frequency range of 5-15 MHz, both manufactured by GE Healthcare, General Electric Company.

The ultrasound images were assessed and measurements were obtained using the MicroDicom software, version -3.1.4.

**Study design**
The ultrasound examination was performed to assess bilateral masseter and temporal muscle thickness (thickest part and at one standardized location for each muscle) as a surrogate measure of total muscle blood flow because of the theory regarding pain due to muscle ischemia as mentioned before. The sites measured were the anterior portion of the origin of the temporal muscle along the hairline and across the origin of the masseter muscle. For the last 12 of the ultrasound examinations, the bulk of the masseter muscle was added as a measuring site. None of the sites were marked on the individuals. These assessments were performed at rest and while clenching before and immediately after the chewing exercise described below, three times respectively to obtain an average measuring result.

**Chewing exercise**
Before the chewing exercise the individuals were asked to answer three questions regarding their pain, discomfort and fatigue in the masticatory muscles, rating it on a Numeric Rating Scale (NRS) of 0 to 10 where 0 was no pain, discomfort or fatigue at all and 10 was the worst imaginable pain, discomfort or fatigue. The subject would then chew three paraffin wax chewing gums without taste in a frequency of 90 Hz (90 chews per minute) for 5.0 minutes. After the exercise the research individual was once again asked to rate their muscle pain, discomfort and fatigue according to the same scale as before.

**Measurements**
The measurements were all performed by one of the examiners to avoid inter-observer differences.
**Outcome variables**
The possibility to assess changes in muscle thickness before and after chewing on a paraffin gum for five minutes and 2) the possibility to assess differences in muscle thickness at rest and upon clenching.

**Statistical methods**
Parametrical statistics were used where applicable and non-parametrical statistics were used elsewhere, both for descriptive and analytical statistics and calculated using the software Stata version 15.1. For calculation of the significance of changes in muscle thickness from rest to clenching as well as between before and after chewing task, the Student’s paired t-test was used. For calculation of the significance of differences between groups regarding pain intensity, muscle fatigue and discomfort, the Wilcoxon matched-pair signed-ranks test was used. A probability level of \( P < 0.05 \) was considered significant.

**RESULTS**
Forty-eight measurements were collected from each of the 22 research individuals; three measurements during rest and three measurements during clenching from each muscle bilaterally, before and after the chewing exercise. For 12 of the individuals the body of the masseter muscle was also assessed, which gave another 24 measurements (Table 3).

The mean thickness of the measured sights increased after the chewing exercise compared to before the chewing exercise, in both rest and during clenching, although none of the results were significant (\( P: >0.05 \)) as seen in table 3. Figure 3 and 4 show the muscle thicknesses for each muscle during rest and during clenching, before and after the chewing exercise.

**Table 3.** Assessment of muscle thickness by ultrasound before and after a 5-min chewing exercise and at rest and during clenching in 22 healthy individuals.

<table>
<thead>
<tr>
<th>Site</th>
<th>Before</th>
<th></th>
<th></th>
<th>After</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
</tr>
<tr>
<td><strong>Temporal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>12.3</td>
<td>2.8</td>
<td>22</td>
<td>13.1</td>
<td>2.4</td>
<td>22</td>
<td>0.296</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clenching</td>
<td>12.9</td>
<td>2.7</td>
<td>22</td>
<td>14.4</td>
<td>3.9</td>
<td>22</td>
<td>0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( P )</td>
<td>0.486</td>
<td>0.205</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Masseter origin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>13.8</td>
<td>2.6</td>
<td>22</td>
<td>14.7</td>
<td>2.2</td>
<td>22</td>
<td>0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clenching</td>
<td>17.3</td>
<td>2.0</td>
<td>22</td>
<td>17.9</td>
<td>1.8</td>
<td>22</td>
<td>0.301</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( P )</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Masseter body</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>12.4</td>
<td>2.1</td>
<td>12</td>
<td>13.7</td>
<td>2.2</td>
<td>12</td>
<td>0.174</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clenching</td>
<td>16.4</td>
<td>2.2</td>
<td>12</td>
<td>17.5</td>
<td>2.3</td>
<td>12</td>
<td>0.264</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( P )</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD: Standard Deviation (mm), \( n \): number of research individuals, \( P \): probability level
In Table 4, muscle pain intensity, muscle fatigue and discomfort are reported. All of the values increased significantly after the chewing exercise ($P: <0.05$). Muscle fatigue was also significantly increased after the chewing exercise ($P: <0.001$).
Table 4. Pain intensity, muscle fatigue and discomfort before and after a five-min chewing task

<table>
<thead>
<tr>
<th>Variable</th>
<th>BEFORE</th>
<th></th>
<th></th>
<th>AFTER</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>25</td>
<td>75</td>
<td>n</td>
<td>Median</td>
<td>25</td>
<td>75</td>
<td>n</td>
</tr>
<tr>
<td>Pain intensity</td>
<td>0-10</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>22</td>
<td>0.0</td>
<td>0.0</td>
<td>75</td>
</tr>
<tr>
<td>Muscle fatigue</td>
<td>0-10</td>
<td>0.0</td>
<td>0.0</td>
<td>1.0</td>
<td>22</td>
<td>2.5</td>
<td>2.0</td>
<td>4.75</td>
</tr>
<tr>
<td>Discomfort</td>
<td>0-10</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>22</td>
<td>0.0</td>
<td>0.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Pain intensity, muscle fatigue and discomfort were assessed on a 0-10 numerical rating scale. 25: the 25th percentile, 75: the 75th percentile, n: number of research individuals, \( P \): probability level.

DISCUSSION

This study shows that assessment of muscle thickness of the masseter and temporal muscles with ultrasound is feasible since the method is capable of detecting differences in muscle thickness between rest and clenching both before and after a chewing task regarding the masseter muscle.

The ultrasound technique, as used in the present study, was able to detect muscle thickness changes between rest and clenching but not between before and after a five-min chewing task. The muscle thickness was larger during clenching compared to at rest. This result is expected since an isotonic contraction were performed when the research individuals clenched. When an isotonic contraction occurs, the muscle fibers shortens in length but increases in thickness which means that the muscle thickness also increases. Our study could not detect corresponding increases in thickness in the temporal muscle. This may be explained by methodological issues discussed below. (18)

There were only tendencies for an increased muscle thickness after the chewing task, however not significant in our study. This could be because of the method used for the examinations, which is discussed below. Because this is a proof-of-concept study, there was no specific expected result regarding muscle thickness after chewing.

Muscle pain intensity, fatigue and discomfort increased after the chewing task in the healthy individuals. The increase in fatigue must be considered as expected but also pain and discomfort increased. However, the pain intensity and discomfort after the chewing task was very minor. If the theory of muscle pain due to ischemia is correct, the results regarding pain and discomfort are also plausible.

Methodological considerations

Ultrasound assessment of tissue thickness, configuration and pathology is to a large extent operator-dependent for maximum accuracy. The operator in this study was fairly...
inexperienced at the beginning of the study but trained for the protocol before the first measurement. There was no systematic change in the reliability of muscle tissue thickness from the first measurement to the last so the results were not influenced by this factor.

The probe connected to the ultrasound has a marking on it that tells the user which way is up and down. This was not noticed by the users in this study before all the examinations where performed. This led to the fact that some of the collected ultrasound images were obtained upside-down. Since the method of examination included a research protocol that started on the right side with the temporalis muscle and ended on the left side with the masseter muscle there was no risk of mistaking which image belonged to which muscle if following the protocol. If an external examiner would assess the ultrasound imaging without knowing about the protocol a confusion would most likely occur. Therefore, the marking on the probe should have been used during the ultrasound examinations.

Another source of error related to the probe could be the angle in which the probe was placed at the measuring site. This could affect the image in the way that the muscle is viewed in a diagonal position rather than in a perpendicular position, which could lead to the muscle giving an appearance to be thicker than it actually is. The same angle of the probe should be used on all the measuring sites, otherwise the results could be affected due to an incorrect muscle thickness measuring. This could have been better accounted for by a stricter methodology but we do not consider this fact to influence the result to a significant degree.

The executors noticed while analysing and measuring the collected ultrasound data that some of the images were of poor quality. This could be due to the untrained executors who performed the ultrasound imaging. It could also be due to the measuring site. Since the temporalis muscle was examined at the origin of the anterior part of the muscle, the clenching imaging could be affected due to the temporalis muscle contracting. When the origin of the temporalis muscle contracts the muscle becomes more convex which leads to the flat surface of the probe partly losing its contact with the muscle. The areas that lose their contact between the probe and the muscle turn out black in the image. This will result in a difficulty measuring the same specific site before and after clenching when assessing the images on the computer. The results of the data collected from the temporalis muscle therefore give a slightly less accurate result than the data collected from the masseter. This is also shown in the results in Figure 4 since one of the research individuals stands out more than the others in measurement of clench after the chewing exercise. This could be because of a wrongful measuring due to difficulties using the same measuring site before and after clenching.

To strengthen the accuracy of the data collected from the temporalis muscle another measuring site could be considered. Since the bulk of temporalis is positioned posteriorly of the hairline of most individuals, the research individuals would then have to shave their hair since the hair could affect the ultrasound imaging negatively. This is not practically or perhaps ethically feasible.

Another subject that can be discussed is the significance collected statistical analysis off the temporal muscle measured after the chewing exercise, before and after clenching. It could be that a swelling of the temporal muscle could occur because of the chewing exercise, therefore the thickness of the muscle does not increase during clenching. This leads to a less reliable result.
During the questioning of the research individuals, more questions could have been asked by the examiners. As an example, questions of earlier history of temporomandibular disorder could have been asked. If there would have been signs of earlier temporomandibular disorders it could be argued that the temporomandibular muscles already have an enlarged volume. A parafunction leading to permanent strain of the muscle could result in an increase of muscle thickness in the same way exercise does, although this is just a hypothesis. The capacity of these muscles could still be lower than an unaffected muscle.

Since the chewing exercise consisted of chewing on a paraffin chewing gum, i.e. a fairly soft chewing gum, the research individuals could have been asked if they regularly chewed on chewing gum. If so, the results could be affected in the way of the muscles not getting tired as easily as an untrained muscle. The use of paraffin for the chewing exercise might not have been sufficient enough to affect the muscle in a way that was noticeable for the subjects. A longer duration or a different chewing gum should be considered in a future study.

Both sex and age were collected from each research individual. It could be hypothesized that muscle thickness could differ between sexes, since men have more muscle mass than women (22), and ages, due to natural muscle atrophy with age (sarcopenia) (23). This would be interesting to investigate further.

Result considerations

When comparing the muscle thickness in resting versus clenching, both before and after the chewing exercise, the results were that the muscle is thicker when clenching than when resting. This result is expected since an isotonic contraction were performed when the research individuals clenched, and the ultrasound image was taken. When an isotonic contraction occurs, the muscle fibers shortens in length but increases in thickness which means that the muscle thickness also increases. (18) The results were significant for the measuring sites for the masseter but not for the temporal muscle which could be explained by the difficulty to interpret and measure the images, the reason which is discussed above.

This study could not determine a difference in muscle thickness between before and after chewing. This could be because of the ultrasound method used for assessment of muscle thickness, a method that is operator-dependent to a great extent. Because this was a proof-of-concept study, there was no expected specific result. If further studies are to be executed, the criteria of the research individuals could be stricter or more elaborate and the information collected about the individuals could be more extensive, as also discussed above.

The difficulty in measuring the thickness of the temporal muscle using ultrasound when clenching led us to believe that comparison of muscle thickness before and after the chewing exercise would not be relevant. In future studies, this has to be methodologically considered beforehand.

The chewing exercise caused muscle fatigue. Like any form of training, a certain level of muscle fatigue is to be expected. If the theory of muscle pain due to ischemia is correct, the results regarding pain and discomfort are also plausible.
Conclusion
This study shows that assessment of muscle thickness with ultrasound is feasible for the masseter muscle since the method is capable of detecting differences between rest and clenching.
REFERENCES

8. Dworkin SF, Leresche L. RESEARCH DIAGNOSTIC CRITERIA FOR TEMPOROMANDIBULAR DISORDERS: REVIEW, CRITERIA, EXAMINATIONS AND SPECIFICATIONS, CRITIQUE.
APPENDIX 1. RESEARCH PROTOCOL
Forskningsprotokoll ”Blodflöde i käkmuskulatur”

Allmänt
Kodnummer
Datum, undersökning
Födelseår
Biologiskt kön
☐ Man ☐ Kvinna
Mediciner:

Inklusion
1. Gör det ont i tinningen, ansiktet, käklederna eller käken en gång i veckan eller oftare?
☐ Ja ☐ Nej
2. Gör det ont när du gapar eller tuggar en gång i veckan eller oftare?
☐ Ja ☐ Nej
3. Har du låsningar eller upphakningar i käken en gång i veckan eller oftare?
☐ Ja ☐ Nej
Inklusionsbeslut, person inkluderas i studien
☐ Ja ☐ Nej

Samtycke
Jag samtycker till att delta i studien efter att ha blivit tillräckligt informerad
☐ Ja ☐ Nej

Underskrift

Namnförtydligande


## Ultrasundersökning

Vilken sida tuggar du oftast på?  
- [ ] Höger  
- [ ] Vänster  
- [ ] Båda sidor lika ofta

### FÖRE TUGGNING

<table>
<thead>
<tr>
<th></th>
<th>M. masseter</th>
<th>M. temporalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Film av blodflöde med doppler</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>Bild i vila</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>Bild vid sammanbitning</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
</tbody>
</table>

Hur mycket smärta har du i käkarna?  
(0 = ingen, 10 = värsta tänkbara) ________

Hur mycket trötthet har du i käkarna?  
(0 = ingen, 10 = värsta tänkbara) ________

Hur stort obehag har du i käkarna?  
(0 = ingen, 10 = värsta tänkbara) ________

**Försöksperson tuggar tre paraffinbitar i takt av metronom (1 Hz) i fem minuter på den sida som person tuggar oftast på**

### EFTER TUGGNING

<table>
<thead>
<tr>
<th></th>
<th>M. masseter</th>
<th>M. temporalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Film av blodflöde med doppler</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>Bild i vila</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>Bild vid sammanbitning</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
</tbody>
</table>

Hur mycket smärta har du i käkarna?  
(0 = ingen, 10 = värsta tänkbara) ________

Hur mycket trötthet har du i käkarna?  
(0 = ingen, 10 = värsta tänkbara) ________

Hur stort obehag har du i käkarna?  
(0 = ingen, 10 = värsta tänkbara) ________
Hej!

Du får denna information eftersom du har anmält intresse av att delta i forskningsprojektet som innefattar mätning av käkmusklers tjocklek och blodflöde med hjälp av ultraljud.

Du får nu en besökstid och denna information inför undersökningen. Detta är första ledet i projektet och kommer att innefatta information om projektet och tre korta frågor. Därefter kommer du att få information om det är möjligt att delta i projektet. Skulle det vara så gär vi vidare med ultraljudsundersökningen.

Om du efter att du fått denna information fortfarande är intresserad av att delta i vår studie kommer du att få signera att du samtycker till personuppgiftshantering samt hur utredningen kommer att gå till.

Med vänliga hälsningar

Tandläkarstuderande Amanda Carlsson och Jennifer Mattsson
Amanda: +46768262100 amanda.carlsson@gmail.com
Jennifer: +46725112998 jenniferMattsson@hotmail.com
Forskningspersonsinformation avseende projektet

Käkmusklers tjocklek och blodflöde undersökt med ultraljud – en pilotstudie
Du tillfrågas om att delta i denna studie.

Syfte
Syftet med denna studie är att jämföra käkmusklers tjocklek och blodflöde hos friska i vila och efter tuggning.

Bakgrund
En av de mest negativa och kraftfulla känslor som en människa kan uppleva är smärta. Ett smärtproblem som varar längre än tre månader är kategoriserat som långvarig smärta. Långvarig smärta i käkregionen kan minska käkens funktion såsom att tala, äta och uttrycka känslor. Att inte kunna göra det på grund av långvarig smärta kan därför ha stor inverkan på livskvalitet och dagliga aktiviteter. Långvarig orofacial smärta kan i viss mån hota överlevnaden hos en människa genom att äventyra matintag och kommunikation.

En av de föreslagna mekanismerna bakom käkmuskelsmärta är lokal syrebrist i muskelvävnaden orsakad av en brist på blodtillförsel på grund av att muskeln drar ihop sig. Detta har inte bevisats ännu, men muskelsmärta kan bero på en förändring i kroppens sätt att reglera blodflödet i muskeln. Användningen av en Doppler-ultraljud för att mäta blodflödet i muskeln är en rutinundersökning som inte tidigare har använts för att mäta muskelblodflöde i tuggmuskler i relation till smärta. Dessutom är ultraljudsundersökningen säker, kräver inget ingrepp och har en stor klinisk potential.

Förfrågan om deltagande
Härmed tillfrågas Du om deltagande i studien. Du har via annons eller tillfrågan anmält intresse av att delta i ovan nämnda projekt som avser att undersöka om ultraljud kan användas som diagnostisk metod för käkmuskelsmärta.

Hur går studien till?
Före undersökningen kommer alla individer att svara på frågeformulär. En undersökning kommer sedan att utföras som består av en undersökning med ultraljudsapparat för att mäta två stora käkmusklers tjocklek och blodflöde, före och efter att du fått utföra en kort tuggövning.

Vad finns det för risker?
Komplikationsrisken för denna undersökning är liten men det finns en viss risk för triggande av smärta, som i så fall kan känna under en övergående period.

Vad finns det för fördelar?
Du kommer bli medveten om eventuella käkleds- eller käkmuskelproblem och bli hänvisad till vård, om så önskas.
Hantering av data och sekretess
Varje forskningsperson kommer att tilldelas ett kodnummer. Dina svar och dina resultat kommer att lagras i kodad form och behandlas så att obehöriga inte kan ta del av dessa. All forskningsdata förvaras inlåst och i kodad form. Tillgång till kodlistan har enbart projektansvarig Per Alstergen och medarbetare, Amanda Carlsson samt Jennifer Mattsson, i projektet.

Hur får jag information om studiens resultat?
Resultatet av studien kommer inte att skickas ut individuellt till deltagande men det färdiga arbetet kommer att publiceras på Malmö University Electronic Publishing (MUEP), som är Malmö Universitets förvaring av vetenskaplig produktion. Denna databas är öppen för alla. Alla resultat kommer redovisas på gruppnivå och det kommer inte vara möjligt att härleda information till enstaka individer.

Ersättning
Någon ersättning för deltagande i studie kommer inte att utdelas.
Undersökningen och information om eventuella diagnoser utförs utan kostnad.

Frivillighet
Deltagandet i studien är helt frivilligt och du kan när som helst, utan att behöva ange skäl för detta, avbryta ditt deltagande. Om du väljer att avbryta kommer dina personuppgifter samt undersökningsresultat att strimlas och inte användas vidare i studien.

Projektansvarig
Per Alstergren
Professor och övertandläkare
Orofaciala smärtsheten, per.alstergren@mau.se

Mer information
Amanda: +46768262100 amanda.carlsson@gmail.com
Jennifer: +46725112998 jennifer.mattsson@hotmail.com
Friska personer sökes till forskningsprojekt om käkmuskler!

Vi söker dig som är smärtfri i käkar och tinningar.

Vi är två tandläkarstudenter som gör vårt masterarbete inom ämnet orofacial smärta och käkfunktion.

Syftet med vårt arbete är att utveckla en metod att undersöka blodflöde i tuggmusklar med hjälp av ultraljud, före och efter tuggning.

För att delta behöver du kunna svenska och vara mellan 18 och 75 år.

Intresserad? Kontakta oss:
Amanda Carlsson  amanda.carlsson@gmail.com
Jennifer Mattsson  jenniferMattsson@hotmail.com

Handledare: Per Alstergen, professor, övertandläkare
Avdelningen för Orofacial smärta och käkfunktion
Odontologiska fakulteten, Malmö Universitet