Endogenous pain control in female TMD patients, before and after conservative treatment: A clinical study

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Summary

Aim
To investigate the hypothesis that the endogenous pain inhibitory system is deficient in patients with myofascial TMD, but that it is restored after successful conservative treatment. A second aim was to investigate if successful treatment changes psychosocial variables.

Methods
Two groups of subjects underwent two sessions of tests. Participants in the test group were diagnosed with myofacial pain according to the Diagnostic Criteria for TMD (DC/TMD) and the control group consisted of healthy subjects with no TMD problems. In each session the RDC/TMD questionnaire and the perceive stress scale (PSS) were completed. The Pressure Pain Threshold (PPT) over the masseter muscle was assessed (test pain) and conditioned pain modulation (CPM) was induced by immersion of the hand into an ice water bath to activate the pain inhibitory system (conditioning pain). Saliva cortisol was measured before and after CPM. In the test group, the first session was before treatment and the second session after successful with occlusal appliance therapy

Results
The PPT showed a significant time effect during the experiment at both visits for both groups (P < 0.001), and a group difference but no interaction between group and time. The post hoc test showed that the PPTs were increased during CPM (P < 0.001) in both groups, i.e both groups showed normal activation of their pain inhibitory systems and that the patients had lower PPTs at all time points than controls. The cortisol level did not change in response to the CPM. There was no difference between groups in scores
for depression and PSS at baseline, but patients had higher level of unspecified physical symptoms than controls (P = 0.007). There was no significant change in level of depression or unspecified physical symptoms after treatment in patients or with time in controls. However, there was a significant decrease in PSS score in the patient group after treatment (P = 0.031).

Conclusions
Our results indicate that patients with TMD have a normally functioning endogenous pain inhibition system; both before and after successful treatment. Levels of depression and unspecified physical symptoms were higher than normal in the patients, but did not change significantly after treatment. The PSS score, decreased after treatment indicating the patients were less affected by psychological stress.
Authors’ contributions

Under supervision by DDS Malin Ernberg, Malin Svensson and Caroline Widegran have both done the following:

We have designed the structure of the clinical examinations. Before the clinical examinations started we discussed the aim of the study, selected the PSS and RDC/TMD axis II questionnaire and made the protocol. We enrolled participants to the patient group and the control group among patients referred to the Department of Dental Medicine at Karolinska Institutet in Huddinge and by displaying posters at Karolinska Institutet and Södertörns Högskola.

While the clinical examinations were done we studied relevant literature for the project, worked with the introduction/background, defined the study material and the selection of methods.

After clinical examination data were transferred to a Microsoft Office Excel document. Data were tabulated with help by Terese Svensson and the results were discussed in relation to existing literature. Finally a manuscript was written.

Caroline Widegran and Malin Svensson have contributed equally in collecting patients, testing the participants, processing the test data, writing and finalizing the manuscript.
Introduction

Tempomandibular disorder (TMD) can be defined as a group of orofacial disorders characterized by pain in the preauricular area, tempomandibular joint (TMJ) or muscles of mastication, limitations/deviations in mandibular range of motion and TMJ sounds during jaw function (American Dental Association, 1983). TMD may be divided in two different syndromes based on its cause: myogenous (muscle related) and arthrogenous (joint-related). The myogenous is more common (1).

TMD and pain

TMD is, as said by the definition, connected to a pain situation. Pain can be described as a subjective experience that is a direct response to tissue damage, such as injury or inflammation. There are also pain conditions caused by disability and distress, these are not directly connected to tissue damage, so called idiopathic pain. This type of pain is relatively common and we know less about it (2).

Myogenous related TMD is most often connected with myogenic pain or tension in the perauricular area. Myogenic pain is related to the origin of muscle cells or fibers. This kind of pain can ensue from contraction of a muscle which leads to reduce oxygenation in the structure. Only contraction cannot lead to pain but together with reduced oxygenation pain arise. When a muscle contracts the intramuscular pressure rise which leads to restriction in blood supply and reduced oxygenation. This can lead to ischemia and pain which can be explained by a gathering of pain producing substances, like bradykinin and serotonin. (3)
Myogenic facial pain is associated to the masticatory systems and is often seen as a symptom of TMD. The masticatory system is complex and involves a group of different muscles and structures. The myogenic facial pain is mostly initialized by chewing or other movements of the jaw. The cause of myogenic facial pain is not established but it appears that it is multifactorial (4). As a result of hyperactivity in the temporo-facial areas and sensitization of nociceptors in one/some/all the masticatory muscles pain can arise (5). The temporal muscle, masseter muscle, medial pterygoid muscle and lateral pterygoid muscle can possess myofascial trigger points (MTrP) which likely can be considered as a possible site for activation of tenderness and chronic myofascial pain. (5). The definition of MTrP was composed by Travel and Simsons 1992 and described it as hyperirritable points located within a taut band of skeletal muscle or fascia when compressed cause referred pain, local tenderness, weakness in the current muscle or spontaneously occurring pain (6) (7). By identifying the MTrP possibility of, for example, treatment with needling or injection therapy has shown good results (8) (9).

The most commonly used diagnostic classification for TMD at present is the Diagnostic Criteria for TMD (DC/TMD). DC/TMD was before known as Research Diagnostic Criteria for TMD (RDC/TMD) (10), however, during a workshop at the annual meeting of the International Association for Dental Research the diagnostic classifications for TMD was updated to this new version. DC/TMD is more appropriate for routine clinical implementation than previous criteria (11). DC/TMD includes two axes, axis I and axis II. Axis I contains clinical physical examination forms and diagnostic criteria for the most common TMD conditions. These conditions are divided into
three groups - myofascial pain, disc displacement and degenerative joint disorders - each group has underlying subgroups. Axis II includes biobehavioral questionnaires to simplify anamnesis and prognosis.

Criteria for myofascial pain according to RDC/TMD are:

- Reported pain in masticatory muscles; Jay, tempels, face, preauricular area or inside the ear, at rest or function.
- Pain on palpation in at least 3 of 20 sites (10 sits at each side), one of them at least in the same side of the reported pain.

(10)

After acute toothache TMD is the most common cause of facial pain (1). Different studies present different prevalence for TMD. In one study a total of 21.5% of the Dutch adult population perceived some dysfunction, and 44.4% showed clinically assessed signs and symptoms of TMD. In nearly all age groups symptoms of TMD appeared more often in women than in men (12). Tecco et al showed that girls had a significantly higher prevalence of myopain than boys (13).

**Endogenous pain control**

Studies have shown that many patients with chronic pain conditions, e.g. fibromyalgia and osteoarthritis have disturbed endogenous pain control (14). A recent study by King et al indicates that also TMD patients have deficiencies in pain modulation systems (15). Interestingly, a study by Kosek and Ordeberg showed that the deficient endogenous pain modulation system in patients with osteoarthritis was normalized after surgery (16).
**Pain controlling system**

Pain can be inhibited or blocked by other stimulus or psychological processes. Activity in pain pathways in the dorsal horn of the spinal cord can be affected when nerve impulses transfers in nerve cells’ synapses. Nerve cells that do not mediate pain and that are activated by pressure touch or vibration has an inhibiting effect on the transmission of nerve impulses in pain pathway. (3)

Werner and Strang divide the human endogenous pain control in three inhibition systems (17):

- The spinal gate control theory (segmental level)
- The endorphin system (general inhibition)
- Conditioned pain modulation (CPM) (segmental and general inhibition.) (18)

The pain controlling system we investigate in this study is termed CPM. CPM was earlier referred to diffuse noxious inhibitory control (DNIC). Today the DNIC-term is only used in animal based research while CPM is used when describing human pain modulation. (18). CPM is based on the finding that a powerful nociceptive stimulus can inhibit pain in another area. Focusing on the most intense pain component is the CNS’ way of handling and prioritizing pain information, which is a prerequisite for physiological stress response and motor defense processes. (17)

One way to experimentally activate the CPM-system is to induce a tonic pain, to a body part (conditioning stimulus), e.g. by immersion of a limb into ice-cold water and then apply a pain stimulus at a remote site in another segment (test stimulus). This leads to inhibition of the latter pain signals resulting in decreased pain (19). CPM includes both pain amplifying and
inhibitory mechanisms (17). One example of the pain amplifying part of the CPM mechanism is when you sprain your ankle. Then pain impulses from other parts of the body are suppressed and the brain focuses only on the pain in your foot. It has been shown that chronic pain syndromes like TMD can lead to a dysfunction of CPM (20) (15).

**Risk factors for chronic TMD**

In the following part we discuss variables that have an influence on the development of TMD. Risk factors involve genetic-, psychological-, autonomic- and sensitivity factors.

**BRUXISM/PARAFUNCTIONS**

Parafunctional activities as a risk factor for TMD were indicated in a report by Michelotti and colleagues in 2009 (21). They showed more specifically that daytime tooth grinding was a risk factor for myofascial pain. Manfredini et al presented in 2010 a review on relationships between bruxism and TMD (22). In this publication 46 articles concerning bruxism and TMD were studied. The association between bruxism and TMD tend to depend on where in the dentition the tooth wear occurs. Tooth wear in the anterior part of the occlusion is, according to the review of Manfredini and co-workers, is not a major risk factor for TMD. However, clinical bruxism diagnosis has been shown to have a positive association with TMD.

**GENETIC FACTORS**

Genetic factors such as specific genes are seen to influence TMD development. Relations between TMD and the genes HTR2A have been reported. HTR2A encodes for one of the receptors for serotonin (23) and COMT encodes for catechol-o-methyl transferase and helps break down
catechol amines such as dopamine, epinephrine and norepinephrine (24). In 2011 Smith et al displayed new potential genetic risk factors for TMD, including genes encoding glucocorticoid receptors and cholinergic receptors. Smith et al explains further that genetic variations in OPRD1 (opioid receptor) and GRIN2A (glutamate receptor) have an influence in TMD risk. Variations in these genes may result in deficiencies in pain regulatory pathways.

Another consideration to have in mind when discussing genetic risk factors in TMD is that the anti-inflammatory activity of IL 10 can differ between people leading to different response to trauma and stress (25).

The hypothalamic-pituitary-adrenal (HPA) system is the primary endocrine stress axis in humans. HPA system is involved in the development of TMD. The glucocorticoid encoded by the NR3C1 gene is the binding site for cortisol and the major element of the HPA system (25).

AUTONOMIC FACTORS
There are also autonomic risk factors behind TMD to consider. Maxiner et al showed in 2011 that patients suffering with TMD had a dysfunction in autonomic activity. This dysfunction was seen as increased heart rates, reduced heart rate variability and a reduction in baroreceptor sensitivity in TMD patients versus healthy subjects in respond to physical and psychological stressors. No correlations between blood pressure and TMD could be seen (26).

PAIN SENSITIVITY FACTORS
People with TMD showed expressively greater pain sensitivity comparing to people without TMD when exposed to mechanical and thermal pain tests
applied to symptomatic and asymptomatic body sites. This supports the thesis of generalized up-regulation of pain processing in TMD and other chronic pain conditions (27).

For pressure pain thresholds (PPT), the difference in pain sensitivity was more pronounced comparing to cutaneous mechanical and heat sensitivity measures (27).

**PSYCHOSOCIAL FACTORS**
Research indicates that psychosocial factors such as depression, anxiety and stress are associated with TMD. Fillingim et al reported in 2011 that TMD patients show higher levels of psychological and affective distress and they experience greater stress compared to a control group (28). In the same article it is presented that premorbid measure of depression and perceived stress were significant predictors of TMD compared to healthy females. Fillingim et al also discuss that although more research is needed psychological variables like this may be seen as predisposing risk factors of development of chronic pain.

Aggarwal et al found that preexisting health anxiety predicted future development of chronic orofacial pain (29).

TMD patients demonstrate higher levels of depression, somatization and anxiety compared to healthy subjects. This proves that psychological factors may play a predisposing role in combination with reduced level of tolerance to pain and a decreased tolerance to stress (30).
**Cortisol**

By definition stress is “the body's reaction to a change that requires a physical, mental or emotional adjustment or response” (31). Cortisol belongs to the group of glucocorticoids. The glucocorticoids secretion is activated by adrenocorticotropic hormone (ACTH) and is produced in the adrenal cortex. The cortisol level seems to vary during the day and is highest in the morning and lowest during midnight (32). All kind of stress conditions leads to a release of ACTH consequently followed of an increase of cortisol levels in the blood and saliva (3) (33). Cortisol has been termed “the stress hormone”. It is secreted in higher level during the body’s sympathetic response to stress. (34) Levels of cortisol in saliva can be seen as an indicator for response to stress.

It has been shown that patient diagnosed with TMD illustrate up to 50% higher levels of daytime salivary cortisol (35). On the other hand, a Swedish study showed that the cortisol level increase in the beginning of a painful situation but when it becomes chronic the opposite situation arise and a decreased level of cortisol appear (36). Meeus et al did a study of the amount of cortisol before and after CPM in patients with chronic fatigue syndrome (CFS) comparing to healthy subject. For both the control group and the patient group a lower amount of cortisol was presented after CPM (37).

After cortisol enters the saliva from the blood most of it remains unbound. Cortisol levels are not affected by the salivary flow rate and do not degrade by enzymes. It also stays in its basic form during the freeze and thawing processes which make it good and relative easy to analyze. It is also a non-invasive and easy test for the patient to implement (38).
Treatment

The treatment for TMD usually includes medical care, consultations and surgical care (1). Reversible (conservative) treatments are usually recommended. Medical care in TMD treatment can be pharmacological, such as non-steroidal anti-inflammatory drugs (NSAID), e.g. ibuprofen or naproxen, muscle relaxants (diazepam and methocarbamol) and tricyclic antidepressants (TCA). In some cases also injections of botulinum toxin have been advocated. Occlusal appliances are also used as a medical treatment. The use of occlusal appliances has been shown to relieve pain in 70-90% of the patients, although the physiological basis for this is largely unknown (1) (39). Consultation includes e.g. psychological counseling, cognitive-behavioral treatment or physical therapy. Surgical care includes arthrocentesis (collecting synovial fluid from the TMJ), arthroscopic surgery or open surgery etc. (1) (40).
Aims

To investigate the hypothesis that the endogenous pain inhibitory system is deficient in patients with myofascial TMD, but that it is restored after successful conservative treatment. A second aim was to investigate if successful treatment changes psychosocial variables.
Materials and Methods

Collection of data

SUBJECTS
Ten female patients with myofascial TMD were recruited among patients referred to the Department of Dental Medicine at Karolinska Institutet in Huddinge. Ten age-matched healthy female subjects were included as a control group.

The inclusion criteria for patients were: female in-between an age of 20 to 60 with facial pain of > 3 months duration and a pain intensity at worst of > 4 on a 0-10 numeric rating scale (NRS) as well as a diagnosis of myofacial TMD according to the DC/TMD criteria (11). Exclusion criteria were systemic inflammatory joint disease, e.g. rheumatoid arthritis (RA), fibromyalgia or whiplash-associated disorders, neuropathic pain, psychiatric disorders, high blood pressure, pregnancy and use of narcotics.

The inclusion criteria for the control group were: healthy female with an age from 20 to 60 without any pain condition from the mouth, jaw, face, temporal muscle, around or in the ear. All subjects received written as well as verbal information and gave their verbal consent. No ethical trial was done.

METHODS

Questionnaire
At both visits the subjects filled in the RDC/TMD Axis II questionnaire as well as the perceived stress scale (PSS). The RDC/TMD Axis II
questionnaires investigate the subjects’ complex problems of TMD, including mental health, depression level, behavioral and social factors (10) (41). The calculations of the scores from the RDC/TMD Axis II questionnaires are made due to the guidelines develop by Dworkin et al. The graded chronic pain scale (GCPS) is included in the RDC/TMD Axis II questionnaires. GCPS is a validated formulary which describes how the daily activity affects due to the pain situation. The questions are designed as where the NRS and are graded from 0, which means no pain/no obstacle, up to 10 that stands for unbearable pain/impracticable. This part of the questionnaire is divided into two parts. The first describes the patients’ current pain intensity as well as the worst and average pain intensity during the last six months. The characteristic pain intensity (CPI) is calculated as the average score reported by the patient multiplicities with 10, which presents with a value between 0-100. The second part describes the influence of facial pain on daily, social and work activities and during the last six month. The score is calculated as for the CPI. The results presents as a function disability score with different points < 30 = 0 p, 30-49 = 1 p, 50-69 = 2 p, > 70 = 3 p. The subject is also asked to estimate how many days daily activities has been prepossessed because of the pain the last 6 months. Different amounts of days gives different points (0-6 days= 0 p, 7-14 days = 1 p, 15-30 days = 2 p, 31+ days = 3 p). After these calculations a disability point calculates which can vary from 0 to 6. This is then combined with the CPI to describe the grade of functional disability.

Different disability points indicate different grades of disability, which is presented in figure 1.
Functional disability

Low disability
- Low intensity <50, <3DP
- High intensity >50, >3DP

Severe disability
- Moderate disability (3-4 DP) regardless of characteristic pain intensity
- Severe disability (5-6 DP regardless of characteristic pain intensity)

Fig 1. Levels of disability according to the RDC/TMD Axis II questionnaire GCPS

Symptom Checklist 90 revised (SCL-90R) is also included in the TMD Axis II questionnaire. This part include 32 questions with are rated with five alternatives (0 = no, 1 = some, 2 = moderate, 3 = Quite much and 4 = very much) which gives information about signs of depression and somatization levels. After calculation a score of 0 to 4 is received. The degree of depression and somatization classifies as low (< 0.428-0.525 p), moderate (0.428-1.104 p) or high (> 0.857-1.105 p), based on population norms.

At The original English version of the PSS developed by Cohen and colleagues consists of 14 questions. It measures the stress the subjects have perceived during the last month and report if their lives seem to be unpredictable, uncontrollable or overloaded. Each question has four alternatives (0 = never, 1 = almost never, 2 = sometimes, 3 = fairly often, and 4 = very often. (42). The PSS has been translated into different languages and we used A Swedish version on the PSS-14 (43).
Assessment of pain
A 0-10 NRS where 0 = no pain and 10 = intolerable pain was used for pain assessments.

Recording of Pressure pain threshold
The pressure pain threshold (PPT) over the masseter muscles was recorded with an electronic algometer (Somedic Sales AB, Solna, Sweden). The algometer consisting of a pistol grip and a circular rod with a pressure sensitive area embraced 1 cm², at the end of the rod. On the top of the grip a display showed the rate of pressure, measured in kPa. The recording of the PPT was made over the most bulky part of the left masseter muscle by holding the algometer vertical against the skin surface. The subject was asked to press a button, connected to the grip of the algometer, as soon as the pressure sensation became painful. This procedure was repeated three times at every measurement part.

Recording of blood pressure
The systolic and diastolic blood pressures (mmHg) were recorded with a digital sphygmomanometer (Omron M3 Intellisense, Omron Healthcare Co Ltd., Kyoto, Japan) consisting of an inflatable arm cuff and a main unit with digital LCD display. The arm cuff was placed on the subject’s left upper arm.

Saliva sampling and analysis
Whole saliva was collected for measurement of cortisol level. The subject was asked to chew on paraffin and spit out enough saliva to fill up the test tube. The saliva samples were immediately frozen in a -20°C until analysis. For an analysis of cortisol level a commercial enzyme immune assay kit was used (No. 1-3002, Salimetrics LLC. PA USA). The saliva samples were first
thawed and then centrifuged at 1500 x (@3000rpm) for fifteen minutes. The kit has ha sensitivity of <0.003 µg/dL and are the minimal concentration of cortisol.

**Conditioned pain modulation**
To induce CPM a cold pressure test was performed. During this the subject was told to immerse the right hand and wrist, i.e. contra laterally to the test side into a 3°C cold circulating water bath (RW 0525-G, Jeitech CO Inc. Seoul Korea). The subjects were asked to keep their hand and wrist in the water until our measurements were completed.

**Procedure/Testing protocol**
At a separate visit the participants were screened for study suitability. If included they came to the clinic for the test session.

All subjects were tested twice: the patients before and after completed TMD treatment and the controls at similar time intervals as the patients.

The subjects were seated in a relaxed supine position in a conventional dental chair. After completion of questionnaires baseline measurements of BP, PPT and pain intensity in the subject’s right hand were performed and saliva was sampled.

The subject then immersed the whole hand and wrist into the water bath. Twenty seconds after immersion of the hand into the water bath and 10 min after withdrawal of the hand from the water bath, the PPT, BP and pain intensity in the hand were again assessed. A new saliva sample was collected 10 minutes after completion of CPM.

To verify that any change in test pain intensity was due to CPM a control experiment was also performed at the first session. In this the subject
immersed her hand into tepid water (36-38°C), but otherwise the experiment was performed in the same manner. The control experiment was performed on the contra lateral side as the CPM experiment.

Before the CPM the subjects were told that they could discontinue whenever they wanted if it became too painful.

The test session was repeated after successful TMD treatment, defined as a 30% reduction of the worst pain intensity (44).

**Statistical analyses**

Data analyses were made with SigmaPlot for Windows, version 11 (Systat Software Inc., Chicago, IL, USA). The normality of the data was tested with the Shapiro-Wilk’s test. For pain intensity (CPI and conditioning pain in hand) and questionnaires, non-parametric statistics were used since the NRS and CPI as well as scores for the questionnaire is ordinal data. For the other variables parametric statistics were used, since they showed a normal distribution, or could be expected to be drawn from a population with normal distribution.

For statistical analyses of baseline differences between groups in pain intensity and scores from the questionnaires, the Mann-Whitney U-test was used, and for differences between the first and second visit the Wilcoxon’s test was used. The Mann-Whitney U-test was also used to compare levels of pain intensity and scores from questionnaires between groups at visit 2.

Differences in PPT, blood pressure and cortisol levels at baseline, and their changes during the CPM/control experiment were tested for statistical significance with repeated measures ANOVA with the Holm-Sidak method for multiple comparison vs a control group as post-hoc test. In the first
model baseline values at the two visits were compared between groups. In this model group (patients vs. controls) was the independent factor, visits (baseline/visit 1 vs. after treatment/visit 2) the repeated factor, and baseline PPT, blood pressures or cortisol level the dependent factors. In the second ANOVA changes in PPT or blood pressures during the experiment were tested, using group as independent factor, time as the repeated factor (BL, during CPM and 10 min after), and levels of PPT and blood pressure as dependent variables. Separate ANOVAs were performed at visit 1 and visit 2. In the third model the percent changes in PPT and cortisol during CPM were tested using group as the independent factor and visit as the repeated factor. In the last model changes in PPT and blood pressure during the control experiment were tested with group as the independent factor, time (baseline vs. during CPM) as the repeated factor, and PPT or blood pressures (during CPM 37 degrees) the dependent factors.
Results

Subjects
The study flow chart is shown in Fig. 2. It is for total 19 subjects, 10 subjects in the patient group and 9 age matched subjects in the control group were enrolled. Three subjects in the patient group did not complete both test sessions. One subject interrupted the CPM at the first test session because of intolerable pain and was excluded from the study. Two subjects did not receive pain relieve by the treatment and therefore were excluded from the second test session. Thus, data were obtained from 18 participants. The mean age in the patient group was 34.2 years (range 24-52) and in the control group 31.2 years (range 25-42 years). The mean time between the first and second visit was 5 (2-7) months in the patient group and 3 (2-4) months in the control group.

Figure 2. Study flow chart showing patient group containing patients with myofacial temporomandibular disorders and control group containing healthy controls.
Baseline comparisons between groups

The median (range) characteristic pain intensity based on graded chronic pain scale (GCPS) in the patients at baseline was 67 (47-80) compared to 0 (0) in the controls (P < 0.001).

Five patients had low grade of disability (0-2 dp) and 3 had moderate grade of disability (3-4 dp), for one patient there was missing data. None of the controls had any disability points.

Results from the RDC/TMD Axis II questionnaires and the PSS are presented in Figure 3.

One patient had moderate level of depression and three had severe levels. The corresponding numbers of controls were two and three. Five patients had moderate level of unspecific physical symptoms and two had severe levels. The corresponding numbers of controls with unspecific physical symptoms were 0 and 1. There was no difference between groups in levels of depression (Mann-Whitney U-test; P = 0.846), but a significantly higher level of unspecific physical symptoms in the patients than controls (Mann-Whitney U-test; P = 0.007). There was no difference between groups in PSS (Mann-Whitney U-test; P = 0.596).

Figure 3. Results from RDC/TMD Axis II questionnaires, including depression, unspecific physical symptoms, and the perceived stress scale (PSS) before treatment (Bef treatm) and after successful treatment (After treatm) are presented in mean value. Results are based on data collected from 9 patients with myofascial temporomandibular disorders and 9 healthy controls before treatment and 7 patients and 9 controls after treatment.
The PPTs over the masseter muscle were significantly lower in the patients than in the controls (Holm-Sidak method; P < 0.001) which are illustrated in Table 1.

Table 1. Mean pressure pain thresholds (PPT; kPa) before (b), during (d) and after (a) conditioned pain modulation (CPM), before treatment in 9 patients with myofascial temporomandibular disorders and 9 healthy controls and after treatment in 7 patients and 9 controls.

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<th>PPT, before treatment</th>
<th>PPT, after treatment</th>
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<tr>
<td></td>
<td>before CPM</td>
<td>during CPM</td>
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<tr>
<td>Patients</td>
<td>132(23)</td>
<td>164(34)</td>
</tr>
<tr>
<td>Controls</td>
<td>201(51)</td>
<td>253(55)</td>
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There was a small, but insignificant, difference between groups in cortisol levels, with lower concentration in the patients, seen Table 2.

There was no difference between groups in systolic or diastolic blood pressure. (Figure 5)

The subjects were asked to rate the pain in their right hand before CPM. Four patients, but no controls reported pain in the hand; two of these reported an intensity of 1, one 3 and one 5 on VAS.

**Effect of treatment/time in groups**

Two of the seven patients that came for the follow-up reported orofacial pain after treatment. Thus, after treatment the median (range) characteristic pain intensity in the patients was 0 (0-37) which was significantly lower than before treatment (Wilcoxon test; P = 0.031). As at visit 1, none of the controls had any orofacial pain at visit 2.

After treatment one patient had moderate level of depression and one had severe levels. The corresponding numbers of controls were five and zero.
There was no change in level of depression after treatment in the patients (Wilcoxon test; $P = 0.250$), and no change between visits in the controls (Wilcoxon test; $P = 0.203$). Neither was there a difference between groups in levels of depression at visit 2 (Mann-Whitney U-test; $P = 0.260$), which can be seen in Figure 3.

Four patients had moderate level of unspecific physical symptoms, and one had severe levels at visit 2. The corresponding numbers of controls were 3 and 0. The level of unspecific physical symptoms tended to decrease after treatment in the patients (Wilcoxon test; $P = 0.094$), whereas there was no change in the controls (Wilcoxon test; $P = 0.688$). There was a tendency to a higher level of unspecific physical symptoms in the patients compared to the controls at visit 2 (Mann-Whitney U-test; $P = 0.054$). However, the difference between groups was smaller than at baseline (before treatment/visit 1).

The PSS decreased significantly after treatment in the patient group (Wilcoxon test; $P = 0.031$), but also showed a trend to a decrease in the controls (Wilcoxon test; $P = 0.055$), even if the magnitude of the decrease was marginal (Table 1). There was a significant difference between groups in PSS at visit 2 (Mann-Whitney U-test; $P = 0.019$).

The PPT at baseline did not change after treatment in the patients (Holm-Sidak test; $P = 0.450$), but decreased significantly between visits in the controls (Holm-Sidak test; $P = 0.005$). There was no difference in PPT between groups at visit 2 (Holm-Sidak test; $P = 0.604$).

The baseline cortisol levels did not differ between visits and there was no difference between groups or interaction between group and visit.
Table 2. Mean cortisol levels (µg/dL) in saliva before conditioned pain modulation (CPM) and 10 minutes after CPM, before and after treatment. Results are based on data collected from 9 patients with myofascial temporomandibular disorders and 9 healthy controls before treatment and 7 patients and 9 controls after treatment.

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<tr>
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<th>Cortisol, Before Treatment</th>
<th>Cortisol, After treatment</th>
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<tr>
<td></td>
<td>Before CPM</td>
<td>After CPM</td>
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<tr>
<td>Mean Value</td>
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<td>Patients</td>
<td>0.166</td>
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<td>Controls</td>
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<td>Patients</td>
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<td>Controls</td>
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Both the systolic and diastolic blood pressures showed a time effect (ANOVA; $F_{1,14} = 5.811, P = 0.030$ and $F_{1,14} = 4.669, P = 0.049$), with lower levels at visit 2, but there were no differences between groups ($F_{1,14} = 0.163, P = 0.691$ and $F_{1,14} = 0.482, P = 0.497$) or interaction (ANOVA; $F_{1,14} = 3.530, P = 0.081$ and $F_{1,14} = 1.865, P = 0.197$). (Figure 5)

**Effects of conditioned pain modulation**

The PPT showed a significant time effect during the experiment at visit 1 (ANOVA; $F_{2,32} = 21.063, P < 0.001$) which is shown in Figure 4. The post-hoc test showed that the PPT was increased during CPM compared to baseline (Holm-Sidak method; $P < 0.001$). There was a significant difference between groups ($F_{1,32} = 14.798, P = 0.001$), with lower PPTs in the patients at all time points, but no interaction between group and time ($F_{2,32} = 2.191, P = 0.128$).
There was also a significant time effect during the experiment for PPT at visit 2 (ANOVA; $F_{2,28} = 18.166, P < 0.001$), but no difference between groups at baseline ($F_{1,32} = 2.263, P = 0.155$). However, there was an interaction between group and time (ANOVA; $F_{2,28} = 4.296, P = 0.024$). The post-hoc test showed that the PPT was increased during CPM compared to baseline only in the controls (Holm-Sidak method; $P < 0.001$) and there was significantly lower PPT during CPM in the patients ($P = 0.016$) than in the controls, i.e. less effective pain modulation.

The percent change in PPTs during CPM compared to baseline was used as a measure of the effectiveness of the pain modulation. The ANOVA showed a significant time effect ($F_{1,14} = 349.375, P < 0.001$), but no group effect ($F_{1,14} = 3.009, P = 0.100$) and no interaction between group and visit ($F_{1,14} = 2.582, P = 0.130$). The post hoc test showed significantly less effective pain modulation at visit 2 in the patient group ($P < 0.001$).

Figure 4. The mean pressure pain threshold (PPT: kPa) over the masseter muscle recorded before, during and 10 minutes after conditioned pain modulation (CPM). Results are based on data collected from 9 patients with myofascial temporomandibular disorders and 9 healthy controls before treatment and 7 patients and 9 controls after treatment.
Both the systolic and diastolic blood pressure showed a significant time effect during the experiment at visit 1 (ANOVA; $F_{2,28} = 20.010$, $P < 0.001$ and $F_{2,28} = 5.143$, $P = 0.012$, respectively), but there were no group differences ($F_{1,28} = 2.140$, $P = 0.163$ and $F_{1,28} = 3.879$, $P = 0.066$, respectively) and no interaction between group and time ($F_{2,28} = 3.042$, $P = 0.062$ and $F_{2,28} = 1.188$, $P = 0.318$, respectively). The post-hoc test showed significantly higher systolic blood pressure during CPM ($P < 0.001$), whereas the test could not determine which time points that differed for the diastolic blood pressure ($P = 0.058$) which can be seen in Figure 5.

Similar findings were found for the blood pressure during the experiment at visit 2, i.e. a significant time effect (ANOVA; $F_{2,28} = 33.727$, $P < 0.001$ and $F_{2,28} = 5.143$, $P = 0.012$, respectively), but there were no group differences ($F_{1,28} = 0.121$, $P = 0.734$ and $F_{1,28} = 0.574$, $P = 0.461$, respectively) and no interaction between group and time ($F_{2,28} = 3.137$, $P = 0.059$ and $F_{2,28} = 1.384$, $P = 0.267$, respectively). The post-hoc test showed that both the systolic and diastolic blood pressure was significantly increased during CPM ($P < 0.001$).

There was no difference between groups ($F_{1,14} = 0.035$, $P = 0.853$), no difference between visits ($F_{1,14} = 0.886$, $P = 0.363$), and no interaction between group and visit ($F_{1,14} = 0.010$, $P = 0.918$) in percent cortisol response to the CPM. (Table 2)
All subjects experienced high levels of conditioning pain, i.e. pain experienced in the hand during CPM. The median (range) in the patients was 8 (5-10) at the first visit and 8 (8-10) at the second visit. In the controls it was 8 (4-9) and 9 (5-10), respectively. There were no significant differences between visits in any group and no difference between groups at any visit. The pain intensity returned to baseline level 10 minutes after finishing CPM-test for all participants.

Neither the PPT nor the blood pressure changed significantly during the control experiment in any group, and none of the subjects experienced any conditioning pain in the hand.
Discussion

Disturbed endogenous pain inhibition is reported in TMD patients. (15) (45). In our study, however, this was not the case. In spite of their chronic TMD pain of moderate intensity, the PPTs in the patient group increased during CPM, both before and after treatment, i.e. a normal reaction to CPM. However, the increase in PPT during CPM was lower in the patient group than in the controls so a somehow dysfunctional endogenous pain modulation cannot be totally ruled out. It could very well be that the number of participants in this study was too small to be able to detect it even though they had chronic pain. Kosek and Ordeberg presented a study on pressure pain modulation in patients with painful osteoarthritis before and after successful surgery. Their results showed an absence of pain modulation in patients before surgery and a normal modulation of pressure pain sensitivity after surgery, indicating that chronic pain caused the dysfunction in pain modulation (16). However, in our study there was no change in pain modulation after treatment and pain relief. One reason to this could be that we used different criteria for pain relief after treatment. Our criteria were a 30% pain reduction in contrast to total relief as was used in the Kosek et al study. Another reason to the different results could be that they induced CPM with the tourniquet test, while we used cold pressure test. Different stimuli may give different outcome.

The patient group had a lower PPT during all test points. King et al reported that patients with TMD had an increased sensitivity to painful stimuli, which correlates with our results. Previous studies have showed that women with masticatory myofascial pain have a lower PPT than healthy individuals (46) (20). Reumatorid arteritis (RA) can also be seen as a chronic pain condition and has also shown a decreased PPT compared with pain free
individuals (47). It has to be noted, however, that the patients after treatment still showed lower PPT compared to the control group. One explanation to this result can be that it takes quite a longer time for the PPTs to restore to a normal level after successful treatment of a chronic pain condition. To our knowledge no study has reported normalization of PPTs after successful treatment with occlusal appliances in TMD patients. Although, in the study by Kosek and Orderberg PPTs were increased to the same levels as in the controls 6-14 months later after treatment (16). Therefore, the patients may need more time after successful treatment before normal PPT levels can be accomplished.

We want to emphasize the decrease in PPT that occurs when applying a repetitive pressure at the same sites many times. Fredriksson and colleagues showed that the PPT decreased significantly when recorded over the same site 5 times. (48). In our study we used the same site to measure PPT during several measurements, this may affect the results. However, all the participants were exposed for the same number of PPT recordings why this probably did not influence our results.

Furthermore Fredriksson et al reported that PPT in healthy individuals increased, when reassessed after 6 months. On the contrary, we found decreased PPTs when comparing visit 1 and 2 in the controls. The reason for this discrepancy is unclear. However, it has to be mentioned that the time between the visits in our study was less than 6 months.

In relation to earlier studies our report indicates that psychological unhealthiness is associated with chronic TMD (28) (30). As can be seen in the results the unspecific physical symptoms decreased (although not significantly) in the patient group after treatment and approached the level
in the control group, but were still higher. This can be seen as a sign of a better psychological health, although, the time of the second experiment might be too early after treatment to evaluate the psychological parameters. Nevertheless, the PSS did decrease radically in the patient group after treatment which must be observed as an indication for a successful treatment and a conversion to better psychological health. Even the GCPS showed a large reduction after treatment that confirmed the successful result of the conservative treatment. However, the occlusal appliance did not reduce the myofascial pain for all patients. Two patients were excluded from the second session because of less than 30% pain reduction. This shows that occlusal appliance therapy is not 100 percent effective for the treatment of patients with TMD-associated pain, which is consistent with other studies in TMD patients. Daif et al recently showed that 85% of TMD patients improved clinically after occlusal appliance treatment (39), which means that 15% did not benefit from this therapy. This reflects the outcome of our study.

Korszun and colleagues showed a higher concentration of cortisol in saliva in TMD patients compared to healthy individuals (35). On the other hand, Rosmond et al demonstrated lower cortisol production in patients with chronic pain disorders compared to healthy individuals (36). Our patient group had, in relation to Rosmond et al, a lower concentration of cortisol at both visits. It has to be highlighted however, that there is a great diurnal variation of cortisol level (32). The visits in this study were not performed at the same time of the day which can explain our results regarding cortisol level. However, the levels in the two groups are closer at the second visit. This can be seen as an indicator that the patients cortisol levels might have
normalized in agreement with reduced stress level, showed by the reduction in PSS score. The decrease in cortisol level in the control group is difficult to explain, since it was not associated with reduced PSS-score.

Difficulties of collecting participant made the number of participants low which must be taken into consideration when interpreting the results. During our collection of participants several patients were excluded because of different reasons. We did not register the excluded patients and the reasons for exclusion, which is a limitation. By estimate, as many as 20 patients were excluded.

In our study we excluded men which make the results only applicable in women. However, TMD is more common in women (13) and because of the limited number of patients we regarded it relevant to exclude men.

Because of the new important information the experiment can give us, we believe it is ethically justified to induce pain in patients and healthy controls. The study followed the principles according to the Declaration of Helsinki, and all participants gave their verbal consent. They were informed that they could terminate the procedure whenever they wanted without consequences.

**Conclusions**

The results of this pilot study indicates that patients with TMD have a normally functioning endogenous pain inhibition system; both before and after successful treatment. Levels of depression and unspecific physical symptoms were higher than normal in the patients, but did not change significantly after treatment. The PSS score decreased after treatment, indicating the patients were less affected by psychological stress. More
research in larger patient samples is needed to evaluate if pain modulation in chronic TMD patients is deficient, but can be restored after successful treatment.
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Handledarintyg

Som handledare för detta projekt tillstyrker jag att studentens eller studenterna ska examineras eftersom dennes/deras prestation och insats i projektet och att den vetenskapliga rapporten är av tillräcklig omfattning och kvalitet för examination.

2012-04-12

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