ANDREAS DAWSON
EXPERIMENTAL TOOTH CLENCHING
A model for studying mechanisms of muscle pain
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Malmö, Sweden
2013
To my mother and father who taught me not to follow where the path might lead, but to go where there is no path and leave a trail …
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ABSTRACT

The overall goal of this thesis was to broaden knowledge of pain mechanisms in myofascial temporomandibular disorders (M-TMD). The specific aims were to:

- Develop a quality assessment tool for experimental bruxism studies (study I).
- Investigate proprioceptive allodynia after experimental tooth clenching exercises (study II).
- Evaluate the release of serotonin (5-HT), glutamate, pyruvate, and lactate in healthy subjects (study III) and in patients with M-TMD (study IV), after experimental tooth clenching exercises.

In (I), tool development comprised 5 steps: (i) preliminary decisions, (ii) item generation, (iii) face-validity assessment, (iv) reliability and discriminative validity testing, and (v) instrument refinement. After preliminary decisions and a literature review, a list of 52 items to be considered for inclusion in the tool was generated. Eleven experts were invited to participate on the Delphi panel, of which 10 agreed. After four Delphi rounds, 8 items remained and were included in the Quality Assessment Tool for Experimental Bruxism Studies (Qu-ATEBS). Inter-observer reliability was acceptable ($k = 0.77$), and discriminative validity high ($phi$ coefficient $0.79$; $P < 0.01$). During refinement, 1 item was removed; the final tool comprised 7 items.

In (II), 16 healthy females participated in three 60-min sessions, each with 24- and 48-h follow-ups. Participants were randomly assigned to a repetitive experimental tooth clenching task with
a clenching level of 10%, 20%, or 40% of maximal voluntary clenching force (MVCF). Pain intensity, fatigue, perceived intensity of vibration (PIV), perceived discomfort (PD), and pressure pain threshold (PPT) were measured throughout. A significant increase in pain intensity and fatigue but not in PD was observed over time. A significant increase in PIV was only observed at 40 min, and PPT decreased significantly over time at 50 and 60 min compared to baseline.

In (III), 30 healthy subjects (16 females, and 14 males) participated in two sessions at a minimum interval of 1 wk. Microdialysis was done to collect 5-HT, glutamate, pyruvate, and lactate and to measure masseter muscle blood flow. Two hours after the start of microdialysis, participants were randomized to a 20-min repetitive experimental tooth clenching task (50% of MVCF) or a control session (no clenching). Pain intensity was measured throughout the experiment. Substance levels and blood flow were unaltered at all time points between sessions, and between genders in each session. Pain intensity was significantly higher after clenching in the clenching session compared to the same time point in the control session.

In (IV), 15 patients with M-TMD and 15 healthy controls participated in one session and the methodology described above was used. M-TMD patients had significantly higher levels of 5-HT and significantly lower blood flows than healthy controls. No significant differences for any substance at any time point were observed between groups. Time and group had significant main effects on pain intensity.

Qu-A TEEBS, the 7-item evidence-based quality assessment tool, is reliable, exhibits face-validity, and has excellent discriminative validity. Tooth clenching was associated with pain, fatigue, and short-lasting mechanical hyperalgesia, but not with proprioceptive allodynia. It seems that tooth clenching is not directly related to delayed onset muscle soreness. In healthy subjects and in patients with M-TMD, levels of 5-HT, glutamate, pyruvate, and lactate were unaltered after tooth clenching. But 5-HT levels were significantly higher and blood flows significantly lower in M-TMD patients than in healthy controls at all time points. These two factors may facilitate the release, and enhance the effects, of other algesic substances that may cause pain.
“I felt like I’d done three rounds with Mike Tyson…all because I was grinding my teeth in my sleep”, så beskrev en patient som intervjuades av Daily Mail i en artikel där det ökade problemet med överbelastning i käkarna beskrevs, vilket kan leda till tandslitage, muskelsmärta, och frakturer på tandmaterial. Det personliga lidandet, och de ekonomiska kostnaderna för både individ och samhälle är stort. Bruxism innebär en daglig och/eller nattlig tandpressning eller tandgnissling och anges med en förekomst av ca 10-20% i befolkningen.

Tidigare undersökningar har visat att tandpressning och psykologisk stress är vanligare bland patienter med kronisk muskelsmärta i ansiktet jämfört med friska försökspersoner, och anses kunna bidra till kronisk muskelsmärta i ansiktet, så kallad myofasciell temporomandibulär dysfunktion (M-TMD). Det har även föreslagits att bruxism, t ex tandpressning, kan leda till träningsvärk i tuggmuskulaturen. M-TMD är ett smärttillstånd som kan drabba tuggmuskulaturen och är ungefär dubbelt så vanligt hos kvinnor som hos män. Vanligt förekommande symtom är smärta och ömhet i tuggmuskulaturen, men även en reducerad tuggfunktion.

Flera studier har använt sig av experimentella tandpressningsmodeller för att öka förståelsen mellan tandpressning och smärtor i tuggmuskulaturen. I dessa studier har olika stor bitkraft använts vid tandpressningen, vilket resulterar i att det blir svårt att jämföra resultaten från dessa studier och dra slutsatser om vilka tandpressningsmodeller som är de mest optimala.

POPULÄRVETENSKAPLIG SAMMANFATTNING (SUMMARY IN SWEDISH)
Vid tandpressning så kan det bli syrefattigt i tuggmuskulaturen, vilket kan resultera i en frisättning av smärtframkallande substanser, såsom serotonin och glutamat. I tuggmuskulaturen finns det smärtreceptorer som kan aktiveras av dessa substanser. I tidigare studier har man observerat att patienter med M-TMD har en högre halt av dessa substanser i tuggmuskulaturen jämfört med friska individer.


I studie I så utvecklades ett instrument som undersöker kvaliteten på experimentella bruxismstådier, som senare kan användas i en systematisk översikt, så att slutsatser kan dras avseende de mest optima experimentella bruxism modellerna som inducerar en smärta på friska individer som efterliknar den kliniska smärtan som patienter med M-TMD uppvisar.

I studie II undersöktes sambandet mellan tandpressning vid olika bitkraftsnivåer och träningsvärk. Våra resultat antyder att träningsvärk i tuggmuskulaturen inte tycks uppstå efter experimentell tandpressning hos friska individer.

I delstudier III och IV undersöktes frisättning av serotonin och glutamat efter tandpressning hos friska individer och patienter med M-TMD med hjälp av mikrodialys. De huvudsakliga fynden var att vi kunde bekräfta tidigare fynd, att patienter med M-TMD har en högre halt av serotonin i tuggmuskulaturen. Däremot utsändrades dessa substanser inte i samband med tandpressning, varken hos friska individer eller hos patienter.
This thesis is based on the following articles:


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# Abbreviations and Definitions

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<th>Definition</th>
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<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CPI</td>
<td>Characteristic pain intensity</td>
</tr>
<tr>
<td>DOMS</td>
<td>Delayed onset muscle soreness</td>
</tr>
<tr>
<td>HEP models</td>
<td>Human experimental pain models</td>
</tr>
<tr>
<td>HTM</td>
<td>High-threshold mechanosensitive</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>M-TMD</td>
<td>Myofascial TMD</td>
</tr>
<tr>
<td>MVCF</td>
<td>Maximal voluntary clenching force</td>
</tr>
<tr>
<td>PD</td>
<td>Perceived discomfort</td>
</tr>
<tr>
<td>PIV</td>
<td>Perceived intensity of vibration</td>
</tr>
<tr>
<td>PPT</td>
<td>Pressure pain threshold</td>
</tr>
<tr>
<td>Qu-ATEBS</td>
<td>Quality Assessment Tool for Experimental Bruxism Studies</td>
</tr>
<tr>
<td>RDC/TMD</td>
<td>Research diagnostic criteria for TMD</td>
</tr>
<tr>
<td>RR</td>
<td>Relative recovery</td>
</tr>
<tr>
<td>TMD</td>
<td>Temporomandibular disorders</td>
</tr>
<tr>
<td>TMJ</td>
<td>Temporomandibular joint</td>
</tr>
<tr>
<td>TTX-r</td>
<td>Tetrodotoxin resistant</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual analogue scale</td>
</tr>
<tr>
<td>VT</td>
<td>Vibration threshold</td>
</tr>
</tbody>
</table>
INTRODUCTION

Chronic pain is a significant health problem and has large economic consequences for society. Approximately 19% of the European population suffers from moderate to severe chronic pain. Chronic pain has a significant impact on the lives of chronic pain patients, with psychological, behavioral, and social consequences. This implies that chronic pain is a highly complex phenomenon.

Temporomandibular disorders (TMD) is a term used for acute and chronic pain conditions that involve the masticatory muscles, the temporomandibular joints (TMJs), or both. Prevalence ranges from 3% to 15% in the general population, and TMD is twice as usual in females as in males. It is the most common chronic orofacial pain condition and can be subdivided into myofascial TMD (M-TMD), disc displacements, arthralgia, osteoarthrosis, and osteoarthritis.

Myofascial TMD
M-TMD is characterized by symptoms such as self-reported jaw muscle pain and soreness, increased pain intensity during function and palpation, and limited jaw function.

Several factors (e.g., genetic, autonomic, and psychosocial) have been suggested as risk factors for TMD. Self-reported tooth clenching has also been proposed as a risk factor for M-TMD. Little is known about the mechanisms that underlie this condition, but it has been suggested that both peripheral and central mechanisms are implicated in the pathophysiology of M-TMD; for a review see Sessle.
Intense muscle exercise can lead to overload, thereby causing pain; it has been suggested that intense muscle contractions can provoke peripheral release of algesic substances\textsuperscript{12,13}. Muscle overload such as tooth clenching may also be associated with disturbed local blood flow, impaired microcirculation, and development of relative ischemia\textsuperscript{14}. Ischemia can trigger a release of algesic substances such as serotonin (5-hydroxytryptamine; 5-HT), prostaglandins, and bradykinin and also activate and sensitize peripheral muscle nociceptors, thereby causing pain\textsuperscript{15}.

**Bruxism**

Bruxism has been defined as “a repetitive jaw-muscle activity characterized by clenching or grinding of the teeth and/or by bracing or thrusting of the mandible. Bruxism has two distinct circadian manifestations: it can occur during sleep (indicated as sleep bruxism) or during wakefulness (indicated as awake bruxism)”\textsuperscript{16}. Although the American Academy of Sleep Medicine developed the International Classification of Sleep Disorders – operationalized criteria for sleep bruxism\textsuperscript{17} – no such criteria exist for awake bruxism. Therefore, one needs to be developed.

Awake bruxism has a prevalence of approximately 20\% in the general adult population and is reported more frequently in females than in males\textsuperscript{18-20}. Sleep bruxism is considered to be the third most frequent parasomnia, and its prevalence in the general population is approximately 8\%, with no gender differences observed\textsuperscript{21}.

Several factors have been reported to contribute to the etiology of bruxism\textsuperscript{22,23}; these can be classified as peripheral or central risk factors. Peripheral factors (e.g., occlusal morphology) were previously considered to be the most important initiating and perpetuating factors. Recent studies, however, have demonstrated the opposite\textsuperscript{22,24}. Central risk factors can be further broken down into psychosocial and pathophysiological factors; pathophysiological factors include sleep-related factors, neurochemical factors, heredity, and diseases; for a review see Lobbezoo & Naeije\textsuperscript{22}.
**Delayed Onset Muscle Soreness**

It has been suggested that bruxism is associated with delayed onset muscle soreness (DOMS). DOMS is mainly associated with eccentric contractions, but it seems that intense concentric contractions can provoke DOMS. DOMS evolves approximately 8–10 h after muscle exercise and peaks around 48 h. Signs and symptoms associated with DOMS are pain on movement, stiffness, fatigue, and soreness. Other features, which are considered to be secondary to pain and fatigue, include allodynia, hyperalgesia, edema, and significantly increased pain intensity in response to vibratory stimuli at 80 Hz. It has been proposed that eccentric contractions damage muscle fibers, which then promote local inflammation and sensitize primary afferents. Large-diameter mechanoreceptive afferents have also been suggested to participate in the generation of DOMS. Vibratory stimuli at 80 Hz is considered an effective stimulus of mechanoreceptive afferents, and the exacerbation of DOMS by 80-Hz vibrations is called proprioceptive allodynia, which means that non-painful stimuli elicit pain by activating proprioceptive afferents.

**Human Experimental Pain Models**

Human experimental pain (HEP) models are the link between basic science and clinical research. HEP models were developed to improve our understanding of pain mechanisms with the final goal of translating these findings into improved patient care. HEP models can be used for research in both patients and healthy subjects. Different aspects of nociceptive processing and pain perception can be studied using HEP models. But HEP models have limitations. Clinical pain is much more complex than experimental pain due to confounding factors, and HEP models are unable to capture the total complexity of clinical pain.

A HEP model activates the nociceptive system, and healthy subjects transiently become pain patients. Endogenous or exogenous stimuli can activate the nociceptive system. The evoked pain response is then quantitatively assessed so that involved pain mechanisms can be evaluated; for a review see Svensson and Arendt-Nielsen.
Since self-reported tooth clenching is a risk factor for M-TMD, it is important to establish the role of tooth clenching in the pathophysiology of M-TMD and to examine the neurobiological and physiological features of jaw muscle pain. To investigate the association between bruxism and M-TMD, several HEP models that mimic bruxism activity have been developed and consist typically of concentric or eccentric contractions. Table 1 lists examples of various experimental bruxism tasks. Concentric contractions can be either dynamic or static for the jaw-closing muscles and resemble tooth clenching. Low to moderate levels of jaw pain develop after such muscle exercises, and it has been suggested that concentric contraction with insufficient relaxation can provoke pain, probably with the same mechanisms observed in ischemic pain. Figure 1 depicts experimental tooth clenching.

**Quality Assessment of Experimental Bruxism Studies**

Systematic reviews are one of several cornerstones in evidence-based medicine. A systematic review is a compilation of all published research over a defined time period that addresses a carefully formulated question. Results of included studies are collected based upon predetermined inclusion and exclusion criteria; data are then critically analyzed and synthesized so that evidence-based conclusions on the benefits or disadvantages of a certain treatment or test of the issue at hand can be drawn.
Table 1. Various experimental bruxism tasks.

<table>
<thead>
<tr>
<th>1st Author/Yr</th>
<th>Muscle exercise</th>
<th>Contraction level</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farella 2010</td>
<td>Clenching</td>
<td>7.5, 10, 15, 25, 40% MVCF</td>
<td>Until exhaustion</td>
</tr>
<tr>
<td>Torisu 2007</td>
<td>Clenching</td>
<td>10% MVCF</td>
<td>Continuously for 30 min</td>
</tr>
<tr>
<td>Hedenberg-Magnusson 2006</td>
<td>Clenching</td>
<td>50% MVCF</td>
<td>15 trials of 30 sec of clenching</td>
</tr>
<tr>
<td>Glaros 2004</td>
<td>Clenching</td>
<td>&gt;10 µV and ≤2 µV</td>
<td>20 min/d for 5 d</td>
</tr>
<tr>
<td>Svensson 2001</td>
<td>Clenching</td>
<td>10% MVCF</td>
<td>60 min</td>
</tr>
<tr>
<td>Arima 1999</td>
<td>Grinding</td>
<td>50 MVCF</td>
<td>9 trials of 5 min of grinding</td>
</tr>
<tr>
<td>Plesh 1998</td>
<td>Clenching</td>
<td>100% MVCF</td>
<td>Intermittent, ramp, and sustained clenching until pain tolerance</td>
</tr>
<tr>
<td>Svensson 1996</td>
<td>Clenching</td>
<td>25% MVCF</td>
<td>15 min of clenching/d for 5 d</td>
</tr>
<tr>
<td>Clark 1991</td>
<td>Protrusion</td>
<td>25, 50, 75, 100% MVCF</td>
<td>Until pain tolerance</td>
</tr>
<tr>
<td>Clark 1989</td>
<td>Clenching</td>
<td>100% MVCF</td>
<td>Until pain tolerance</td>
</tr>
<tr>
<td>Bowley 1987</td>
<td>Grinding, clenching, protrusion</td>
<td>50 and 70% MVCF</td>
<td>30 min of grinding at 50% MVCF, 5 min of clenching at 70% MVCF, 5 min of protrusion, 22.5 min of clenching at 70% MVCF</td>
</tr>
<tr>
<td>Christensen 1981</td>
<td>Clenching</td>
<td>100% MVCF</td>
<td>Until fatigue, and until pain</td>
</tr>
<tr>
<td>Scott 1980</td>
<td>Protrusion</td>
<td>Vigorously</td>
<td>5 min</td>
</tr>
<tr>
<td>Christensen 1971</td>
<td>Grinding</td>
<td>Grinding so that grinding noises appeared</td>
<td>5 min</td>
</tr>
</tbody>
</table>

A review of selected experimental bruxism studies reveals large variation in the methodology of the experimental bruxism models concerning:

- Type of jaw muscle exercise
- Duration of the bruxism task
- Level of contraction
- Outcome measures
- Number of exercise days
- Number of follow-up days
This makes it difficult to compare the findings of experimental bruxism studies. Those that use a contraction level of 100% MVCF seem to have low external validity. Patients with TMD engage in tooth clenching for longer periods of time\textsuperscript{60}, and since maximal bite force can only be generated for a short period of time\textsuperscript{61}, tooth clenching most likely occurs at lower intensities. Due to the varying standards among published articles, quality assessment could be said to be the keystone of a systematic review. Study results cannot be judged to have a high level of evidence or contribute substantially to a review’s conclusions and recommendations if quality standards defined as lack of bias, applicability, and good reporting and design are not met. There is currently a lack of systematically developed and evaluated tools for assessing experimental bruxism, which has created a need for a platform of measures that can be used in clinical experimental bruxism trials and would allow results to be compared between studies and general conclusions drawn.

**Peripheral Neurobiological Mechanisms of Muscle Pain**

Figure 2 illustrates the receptor molecules on a nociceptive ending. Muscle nociceptors are free nerve endings of $\text{A}^\delta$ and $\text{C}$ fibers. No formal classification of muscle nociceptors exists, but they are generally divided into high-threshold mechanosensitive (HTM) receptors, chemonociceptors, and polymodal nociceptors.
Figure 2. A schematic illustration of receptor molecules and intracellular events in a nociceptive ending. 5-HT, adenosine, and prostaglandin E2 target receptor molecules, and protein kinase A is activated, which increases the sensitivity of Na⁺ channels. Ion influx increases, and the muscle nociceptors become sensitized. Other receptor molecules are purinergic (P2X3) and vanilloid receptors (VR-1). ATP binds to P2X3 while VR-1 receptors are sensitive to protons (H⁺) and heat. SP—substance P; CGRP—calcitonin gene related peptide; GLU—glutamate; KA—kinin acid; AMPA—alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; NMDA—N-methyl-D-aspartate; TTX—tetrodotoxin (modified after Mense65).

Aδ and C fibers supply HTM receptors, which are only activated by noxious pressure stimuli. C fibers have a higher density of HTM receptors than do Aδ fibers. As the name indicates, chemonociceptors are excited by chemical stimuli such as algesic substances and ischemic contractions. Chemonociceptors are mainly supplied by C fibers. Polymodal nociceptors are activated by noxious pressure stimuli and algesic substances. Various algesic substances activate different proportions of C fibers; for example, intramuscular injection with an acidic buffer solution activated 60% of the C fibers, while hypertonic saline activated all C fibers62.
Muscle nociceptors are activated and sensitized by a range of algesic substances, by targeting receptor molecules in the membrane of the nociceptive endings, and thereby causing pain. Voltage-gated ion channels are important for nociception. Two voltage-gated channels – tetrodotoxin-resistant (TTX-r) sodium channels – have been identified on nociceptive primary afferents (Nav 1.8, and Nav1.9). These channels can be sensitized by algesic substances that place these channels in a hyperpolarized state, making them more easily activated by noxious stimuli.\textsuperscript{62, 63}

Substances such as 5-HT, bradykinin, and prostaglandin E\textsubscript{2} activate or sensitize muscle nociceptors. Bradykinin binds to B\textsubscript{1} and B\textsubscript{2} receptors, 5HT binds to 5-HT\textsubscript{3} receptors, and prostaglandin E\textsubscript{2} binds to prostanoid receptors. Other receptor molecules in nociceptive membranes include acid-sensing ion channels (ASIC) type 1 and 3, transient receptor potential vanilloid 1 (TRPV1) receptor, purinergic receptors such as P2X3 receptors, and N-methyl-D-aspartate (NMDA) receptors. Protons activate TRPV1 and ASIC receptors; adenosine triphosphate (ATP), P2X3 receptors; and glutamate, NMDA receptors.\textsuperscript{62}

It is likely that different muscle nociceptors exhibit different receptor molecules. Receptor molecules are most likely inconsistent and the binding properties and the density of a specific receptor molecule can change in response to alterations in the biochemical environment. In healthy muscle tissue, bradykinin ligands to the B\textsubscript{2} receptor, but in inflamed muscle tissue, the number of B\textsubscript{1} receptors are increased in the nociceptive membrane, and bradykinin binds to B\textsubscript{1} instead. Another example is the TRPV1 receptor, which is activated by protons in healthy muscle tissue. But when the pH is reduced due to muscle exercise until exhaustion or inflammation, the density of TRPV1 in the nociceptive membrane is increased and more TRPV1 receptors are activated.\textsuperscript{62} This indicates that receptor molecule density is dynamic and dependent on the biochemical environment.

It seems that some algesic substances enhance the effects of other algesic substances on muscle nociceptors. When bradykinin activates muscle nociceptors, pain is perceived, but in the presence of 5-HT
or prostaglandin E₂, the evoked sensation of pain is higher. This is of clinical interest since these substances are released in response to tissue damage. Initially after tissue damage, the concentrations of 5-HT and prostaglandin E₂ are low, so excitation is unlikely. Instead they might interact with receptor molecules in the membranes of the nociceptive endings, sensitizing the muscle nociceptors and enhancing the effect of other algesic substances that could activate muscle nociceptors.

Thus, algesic substances can sensitize muscle nociceptors by reducing their activation thresholds. Stimuli that normally do not provoke pain might after sensitization elicit pain (alldynia), and stimuli that normally provoke pain can induce increased sensations of pain (hyperalgesia).

Peripheral as well as central mechanisms are involved in the transition from acute to chronic muscle pain; for a review see Mense.

Algesic Substances
In response to tissue damage, inflammation, or ischemia, a cascade of events leads to a release of algesic substances. These substances can sensitize or activate muscle nociceptors. Two neuroactive substances thought to be involved in chronic pain are 5-HT and glutamate.

Serotonin
5-HT is a neurotransmitter that is synthesized in serotonergic neurons in raphe nuclei in the brainstem and in enterochromaffin cells in the digestive tract. 5-HT is synthesized from the essential amino acid tryptophan. Approximately 90% of 5-HT is synthesized peripherally. Mast cells, platelets, and enteric neurons contain 5-HT. Blood contains low concentrations of unbound 5-HT.

It was previously believed that 5-HT was only involved in the descending pain inhibition system, but more recently 5-HT is thought also to participate in the facilitation of pain at the spinal level.
Platelets and neurons release 5-HT in response to tissue trauma and inflammation. Tissue damage evokes axon reflexes, which provoke a release of neuropeptides, such as calcitonin gene-related peptide and substance P. These neuropeptides elicit a release of interleukins and cytokines that degranulate mast cells, and platelet-activating factors are released. Platelet-activating factor stimulates the degranulation of platelets, upon which 5-HT is released. 5-HT can then alter the sensitivity of TTX-r sodium channels via protein kinase A by inducing a hyperpolarization, which increases the activation rate. This could increase the sensitivity of mechanosensitive afferents for other algesic substances and reduce the activation threshold of TRPV1; the result is primary hyperalgesia. 5-HT binds to 5-HT3 receptors, provoking a release of glutamate and substance P in the synaptic cleft between the pre- and postsynaptic neuron, and the pain signal is transmitted to the central nervous system (CNS). But substance P and glutamate may also be released peripherally.

It has been suggested that 5-HT increases the algesic effect of bradykinin, prostaglandin E2, and histamine by lowering the activation thresholds of muscle nociceptors.

**Glutamate**

Glutamate is an excitatory amino acid that is synthesized in the CNS and the peripheral nervous system and is involved in pain modulation at a peripheral and a central level. Glutamate targets NMDA receptors and α-amino-3-hydroxy-5-methyl-5-isoxazolepropionate (AMPA) receptors, which reside on primary afferents. Primary afferents contain glutamate at peripheral and central terminals. Short-lasting, painful stimuli lead to a release of glutamate from the primary afferent to the synaptic cleft. The released glutamate binds to AMPA receptors on the second order neuron, but during long-lasting painful stimuli, glutamate binds to NMDA receptors, which leads to a higher density of NMDA receptors on the second order neuron (central sensitization). Activation of peripheral NMDA receptors might be associated with altered sensitivity of the second order neuron, and pain is perceived.
The Role of 5-HT and Glutamate in M-TMD

Interstitial concentrations of masseter muscle 5-HT and glutamate are significantly higher in patients with M-TMD compared to healthy subjects\textsuperscript{79,80}. Similar results have been observed in patients with trapezius myalgia\textsuperscript{81,82}. Recently, it was investigated whether low-force exercise is associated with increases in trapezius muscle 5-HT and glutamate. An increase in 5-HT was not observed\textsuperscript{82}, but the level of glutamate increased significantly in healthy subjects and in patients with trapezius myalgia\textsuperscript{81}. Although 5-HT and glutamate are involved in pain transmission at central and peripheral levels\textsuperscript{11}, it has not yet been established what mechanisms might cause the higher levels of masseter muscle 5-HT and glutamate observed in patients with M-TMD. One explanation might be that tooth clenching causes a release of these algesic substances due to ischemia.

Intramuscular injections of 5-HT or glutamate in the masseter muscle of healthy subjects are associated with pain, alldynia, and hyperalgesia\textsuperscript{83,84}. 5-HT targets 5-HT\textsubscript{3}, and glutamate targets NMDA receptors, which activate muscle nociceptors, and causing pain perception\textsuperscript{83,84}.

Microdialysis in the Study of Algesic Substances

Microdialysis is one way to study the concentrations of various fluids in body tissue and organs. This technique is similar in function to a capillary blood vessel, where molecules in extracellular fluid diffuse through endothelial cells to blood vessels. A semipermeable probe connected to a microinfusion pump is inserted into the tissue or organ and perfused with a physiological medium. Molecules in the extracellular fluid can passively diffuse back and forth through the membrane, and the medium is sampled\textsuperscript{85}. The cut-off value of the semipermeable probe (the size of the membranes’ pores) determines which molecules diffuse through the membrane and which molecules are prevented from reaching the dialysate\textsuperscript{86}. After the medium permeates the semipermeable probe, the dialysate can be chemically analyzed\textsuperscript{85}. Figure 3 illustrates microdialysis.
Relative Recovery
The dialysate samples do not reflect the true extracellular concentration of the substances of interest. The term recovery describes the concentration in the dialysate in relation to the extracellular concentration. The higher the recovery, the more the dialysate concentration of a specific substance reflects the true extracellular concentration of that substance. Relative recovery (RR) describes the ratio between the true concentration of a substance and the concentration in the dialysate. RR is affected by flow rate, diffusion rate, and membrane type. If RR is known, the interstitial concentration of specific substances can be calculated: $(C_d - C_p)/RR + C_p$, where $C_u$ is the concentration of the substance in the dialysate and $C_p$ is the concentration of the substance in the perfusate.

Figure 3. The microdialysis technique
The specific aims of this thesis were to:

- Develop a tool that could be used to judge the scientific quality of experimental bruxism studies in systematic reviews (I).

- (a) Evaluate the test-retest reliability of vibrotactile sensitivity of the masseter muscle and (b) assess proprioceptive allodynia after tooth clenching exercises (II).

- Investigate the intramuscular release of algesic substances after experimental tooth clenching in the masseter muscle of healthy subjects (III).

- Investigate the effects of experimental tooth clenching on the levels of 5-HT, glutamate, pyruvate, and lactate as well as on pain intensity, fatigue, and pressure pain thresholds in the masseter muscle of patients with M-TMD (IV).
HYPOTHESES

The following hypotheses were tested:

Study II
1. The vibration threshold is decreased after experimental tooth clenching.
2. Intense vibrations exacerbate pain after tooth clenching.
3. Tooth clenching increases pain and fatigue.
4. Tooth clenching decreases pressure pain thresholds.

Study III
1. Experimental tooth clenching elevates masseter muscle interstitial levels of 5-HT, glutamate, pyruvate, and lactate.
2. In response to experimental tooth clenching, females have a significantly higher release of 5-HT, glutamate, pyruvate, and lactate compared to males.

Study IV
1. M-TMD patients have significantly higher levels of muscle 5-HT, glutamate, pyruvate, and lactate than healthy subjects at rest and following experimental tooth clenching.
2. Levels of 5-HT and glutamate are significantly correlated with pain intensity and pressure pain thresholds in M-TMD patients and healthy subjects.
3. M-TMD patients have significantly higher pain intensities and fatigue and significantly lower pressure pain thresholds than healthy subjects at rest and following contractions.
MATERIALS AND METHODS

All studies were conducted at the Department of Orofacial Pain and Jaw Function at Malmö University, Malmö, Sweden and followed the Declaration of Helsinki guidelines. The Regional Ethics Review Board at Lund University approved methods and subject selection (*study II*: 2009/264, *study III* and *study IV*: 2010/31). Before entering the studies, subjects signed an informed-consent form and understood that they could withdraw from the project at any time with no consequences. Subjects in (III) and (IV) received financial compensation upon completion of their participation.

Healthy Subjects (II–IV)
In all, 68 healthy subjects who were recruited among staff at Malmö University participated in the studies. Three subjects in (IIA) participated in (IIB). Subjects were matched according to age and gender in (III). 15 healthy subjects from (III) participated as healthy controls in (IV) and were age- and gender-matched to the M-TMD patients. Tables 2 and 3 list the characteristics of the healthy subjects in (II – IV).

Study II
Inclusion criteria were:

- Good general health
- No orofacial pain complaints
Exclusion criteria were:

- Age ≤ 18 yr
- Male gender
- TMD or other orofacial pain complaints
- Systemic inflammatory connective tissue diseases (e.g., rheumatoid arthritis)
- Whiplash-associated disorder
- Fibromyalgia
- Neuropathic pain
- Use of analgesics (e.g., paracetamol, nonsteroidal anti-inflammatory drugs, salicylate drugs, and opioids) or other medication that would influence pain perception (e.g., anti-depressants and anti-epileptic drugs)
- Pregnancy
- Severe skeletal malocclusion
- Extensive restorations such as fixed partial dentures

Table 2. Characteristics of the healthy subjects in studies II and III, number of subjects (N), gender, and age (yr; mean ± SD).

<table>
<thead>
<tr>
<th>Subjects (N)</th>
<th>Study</th>
<th>IIA</th>
<th>IIB</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td></td>
<td>25</td>
<td>16</td>
<td>30</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td>25</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td>-</td>
<td>-</td>
<td>14</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age (mean ± SD)</th>
<th>Study</th>
<th>IIA</th>
<th>IIB</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td></td>
<td>42 ± 12</td>
<td>32 ± 10</td>
<td>38 ± 17</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td>42 ± 12</td>
<td>32 ± 10</td>
<td>36 ± 16</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td>-</td>
<td>-</td>
<td>41 ± 18</td>
</tr>
</tbody>
</table>
Studies III and IV
Inclusion criteria were:

- Good general health
- No orofacial pain complaints.

Exclusion criteria were:

- Age $\leq 18$ yr
- Systemic inflammatory connective tissue diseases (e.g., rheumatoid arthritis)
- Whiplash-associated disorders
- Fibromyalgia
- Neuropathic pain or neurological disorders (e.g., oromandibular dystonia)
- Pain of dental origin
- Pregnancy or lactation
- High blood pressure
- Anticoagulants
- Ongoing dental treatment
- Extensive restorations (e.g., full-bridges and dentures)
- Allergy to antibiotics, prilocaine, or lidocaine
- Use of analgesics (e.g., paracetamol, NSAIDs, salicylate drugs, and opioids) or other medication that would influence pain perception (e.g., anti-depressants or anti-epileptic drugs)
- Severe skeletal malocclusions

Patients (IV)
All patients were recruited between October 2011 and July 2012 from consecutive patients referred to the Department of Orofacial Pain and Jaw Function at Malmö University. Table 3 presents the characteristics of the patient group.
Table 3. Characteristics of the patients and the healthy subjects in study IV, number of subjects (N), age in yr and duration in mo of M-TMD (mean ± SD). Median (interquartile range) number of muscle sites and joint sites with pain on palpation and characteristic pain intensity.

<table>
<thead>
<tr>
<th>Study IV</th>
<th>M-TMD patients</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (N)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Females</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Males</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>32 ± 13</td>
<td>32 ± 12</td>
</tr>
<tr>
<td>Females</td>
<td>28 ± 8</td>
<td>28 ± 8</td>
</tr>
<tr>
<td>Males</td>
<td>43 ± 21</td>
<td>40 ± 20</td>
</tr>
<tr>
<td>Duration of M-TMD (mo)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>59 ± 60</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Females</td>
<td>66 ± 67</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Males</td>
<td>38 ± 36</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>No. of muscle sites with pain on palpation (max. 20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>14 (8)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Females</td>
<td>15 (4)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Males</td>
<td>6 (2)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>No. of TMJ sites with pain on palpation (max. 4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>1 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Females</td>
<td>1 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Males</td>
<td>1.5 (2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Characteristic Pain Intensity (CPI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>57 (17)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Females</td>
<td>57 (10)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Males</td>
<td>45 (41)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Inclusion criteria for the M-TMD patients were:

- Orofacial pain for at least 6 mo
- A diagnosis of myofascial TMD per RDC/TMD criteria\(^4\), with at least moderate pain upon palpation of the masseter muscles

Exclusion criteria in (IV) were the same as in (III).
**Experimental Protocol**

**Study I**
Streiner and Norman’s 5-step method for developing quality assessment tools – *(i)* preliminary decisions, *(ii)* item generation, *(iii)* face-validity assessment, *(iv)* assessment of inter-observer reliability and discriminative validity, and *(v)* refinement of the final instrument – was followed.

**Study II**
This single-blinded randomized cross-over trial comprised three 60-min sessions with a 24- and 48-h follow-up after each session. Participants were randomly assigned to a tooth clenching exercise with clenching levels of 10%, 20%, or 40% of maximal voluntary clenching force (MVCF). Pain intensity (VAS\_pain), fatigue (VAS\_fatigue), vibration threshold (VT), perceived intensity of vibration (PIV), perceived discomfort (PD), and pressure pain threshold (PPT) were measured throughout the trial. These six measurements occurred at baseline, immediately after each bout of clenching, and at the 24- and 48-h follow-ups. Figure 4 illustrates the design.

*Figure 4. Six bouts of tooth clenching (1 to 6) over 1 h. Each bout lasted 5 min. Contraction level was randomized between sessions (10%, 20%, or 40%). MVCF–maximal voluntary contraction force; VT–vibration threshold; PIV–perceived intensity of vibration; PD–perceived discomfort; PPT–pressure pain threshold; VAS\_pain–pain intensity; and VAS\_fatigue–intensity of fatigue.*

**Study III**
This study was a single-blinded, randomized, placebo-controlled trial, and comprised two sessions. Figure 5 is a schematic illustration of the study design. Before insertion of the microdialysis catheter, MVCF and PPT were assessed. Intramuscular microdialysis was
done in the right masseter muscle to sample 5-HT, glutamate, lactate, and pyruvate. Subjects were randomly allocated to a repetitive experimental tooth clenching exercise or a control session with no clenching.

A 120-min stabilization period directly followed probe insertion, the last 20 min of which served as a baseline measure of the experiment. After stabilization, baseline assessments of pain intensity and fatigue were made, followed by a 20-min clenching (at 50% of MVCF) or control (relaxation of the masticatory muscles) session and 40 min of rest (recovery). Assessments of pain intensity and fatigue were repeated directly after each clenching and control session and after recovery. PPT was measured after removal of the microdialysis probe.

**Study IV**

This study was a case-control study and consisted of one session with a repetitive experimental tooth clenching exercise (at 50% of MVCF). The same data collection made in (III) was also made in (IV). Figure 5 illustrates the study design.

*Figure 5. Schematic illustration of the study design of (III) and (IV). Intramuscular microdialysis was conducted to sample 5-HT, glutamate, pyruvate, and lactate. Two hours after the start of microdialysis, the subjects performed a repetitive tooth clenching task. Pain intensity and fatigue were measured at baseline, after the clenching task, and after recovery. Pressure pain threshold (PPT) was assessed before insertion of the microdialysis probe, and after the removal of the microdialysis probe.*
**Data Collection**

Table 4 defines data collection in the four studies.

*Table 4. Type of data collection in studies I–IV.*

<table>
<thead>
<tr>
<th></th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preliminary decisions</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Item generation</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychometric assessment</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical examination</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Self-reported measures</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Experimental tooth clenching</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Psychophysical examination</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Microdialysis</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical analysis</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Preliminary Decisions (I)**

The steering group (Andreas Dawson, AD; and Susanna Axelsson, SA) defined the desired characteristics and purpose of the quality assessment tool: the tool should *(i)* be suited for use in systematic reviews of experimental bruxism studies, *(ii)* be able to assess the methodological quality of the study in generic terms (relevant to all experimental bruxism studies), *(iii)* be easy to understand and respond to and quick to use, and *(iv)* contain a maximum of 10 items.

**Item Generation (I)**

A search of PubMed was conducted with the following MeSH terms: Masseter Muscle AND Pain Measurement AND Bite Force OR Isometric Contraction AND Masticatory Muscles. A hand search was also made. Following a review of 16 articles, the first preliminary tool was compiled.

**Psychometric Assessment (I)**

*Face-Validity Assessment*

A Delphi procedure was chosen to assess face validity, and 11 experts were asked to participate on the Delphi panel. Only participants with extensive clinical and research experience in this field were considered.
The Delphi Procedure. In the first round, Delphi panelists received the preliminary tool created in Item Generation plus instructions. In each succeeding round, panel members were sent instructions, a tabulated summary of panel member responses and free-text feedback, the revised preliminary tool, and a list of undecided items (the undecided list) from the preceding round. The Delphi members were asked to read the instructions thoroughly and, while keeping in mind the ideal properties of a quality assessment tool, rate the items proposed for inclusion in a preliminary tool on a 5-point Likert scale (1, Strongly Disagree; 2, Moderately Disagree; 3, Neutral; 4, Moderately Agree; 5, Strongly Agree) in round 1 and on a Yes-No-Undecided scale in subsequent rounds. The steering group took all free-text comments made by the Delphi panel into careful consideration before including, excluding, and rephrasing items during revision of the preliminary tool. Panelists were informed of the steering group’s reasoning for the changes in the preliminary tool.

The Delphi procedure continued for a maximum of four rounds or until a consensus was reached on the 10 or fewer items to be included in the quality assessment tool, their meaning, and how to rate them.

Assessment of Reliability and Discriminative Validity
To assess discriminative validity, two investigators reviewed 11 studies randomly chosen from the PubMed search results and the results of the manual hand search. The Journal of Orofacial Pain’s checklist for manuscript review was used to classify the studies as high quality or low quality studies; for details see (I). Two other authors (AD and SA) assessed the same articles using the quality assessment tool for experimental bruxism studies. The results from the two teams were compared and discriminative validity was assessed. Inter-observer reliability was assessed by comparing the results between the two authors that used the quality assessment tool for experimental bruxism studies.

Refinement of the Final Instrument
Based on the results of the reliability and discriminative validity assessments, the instrument was refined.
Clinical Examination (II-IV)
All participants underwent a dental examination and an examination of the masticatory system and of the head and neck. The purpose of this was to confirm the absence of orofacial pain complaints in healthy subjects and a diagnosis of M-TMD in the patients.

Self-Reported Measures (II-IV)
Before participation, the healthy subjects and M-TMD patients completed a comprehensive questionnaire describing pain duration, current pain intensity, worst pain intensity, and average pain intensity. Characteristic Pain Intensity (CPI) was calculated by multiplying by a factor of 10 the mean of current, worst, and average pain intensity.

Two 100-mm visual analogue scales (VAS) were used to assess intensities of pain and fatigue (anchor definitions: no pain/no fatigue and worst imaginable pain/worst imaginable fatigue).

Experimental Tooth Clenching (II-IV)
A bite-force transducer (Aalborg University, Denmark) placed between the molars on the right side assessed MVCF (kg). Three times, subjects were asked to bite down on the transducer (clench) as intensely as possible for 2–3 sec. Mean MVCF was calculated from three registrations.

Psychophysical Examination (II-IV)
Pressure Pain Threshold
An algometer (Somedic Sales AB, Hörby, Sweden) assessed PPT – defined as the amount of pressure needed to produce a sensation of pain – on the right masseter muscle. Upon reaching the PPT, subjects pressed a button to stop stimulation. A constant pressure of 30 kPa/s was applied with a 1.0-cm² probe. The mean of three measurements made at 60-s intervals was calculated. This way of measuring the PPT has acceptable reliability.

Vibration Threshold (II)
The Vibrameter® (Somedic Sales AB) delivered 100-Hz vibratory stimuli to the right masseter muscle with a constant application pressure of 650 g. The stimulating probe was a plastic cylinder with a diameter of 13 mm. Ascending vibratory stimuli were used to make
three assessments of the vibration perception threshold, defined as the amplitude (µm) at which the participant first perceived vibration. Descending vibratory stimuli were used to make three assessments of the vibration disappearance threshold, the amplitude at which vibration was no longer perceived. Means of the three measurements determined the vibration perception and disappearance thresholds. VT was then calculated as the mean of these two thresholds.92

Perceived intensity of vibration and perceived discomfort were assessed with 15-s fixed vibratory stimuli (Vibrameter®, 100 Hz, 399.99-µm amplitude) applied to the right masseter muscle with a constant application pressure of 650 g. Subjects were instructed to rate perceived intensity of vibration and perceived discomfort on 0–50–100 numeric rating scales (perceived intensity of vibration: 0 = no sensation, 50 = pain threshold, 100 = most imaginable pain; perceived discomfort: 0 = no sensation, 50 = discomfort, 100 = most imaginable discomfort).

Microdialysis (III-IV)
Intramuscular microdialysis was done to sample masseter 5-HT, glutamate, lactate, and pyruvate. After the belly of the right masseter muscle was palpated and identified, the skin over this region was anesthetized with topical anesthesia (EMLA® 20 mg/g; AstraZeneca AB, Södertälje, Sweden) for 20 min. A standard catheter (Ø 1.3 x 32 mm, BD Venflon Pro; Becton Dickinson Infusion Therapy AB, Helsingborg, Sweden) was inserted into the center of the masseter belly at a 45° angle to the skin and a depth of 20 mm. The needle was removed, and the catheter was retracted 10 mm, leaving 10 mm of plastic within the muscle. The catheter was cut 10 mm from the skin surface.

A sterile and flexible microdialysis probe (Ø 0.5 mm; membrane length 10 mm; shaft length 20 mm; molecular cut-off: 6 kDa, MAB11.20.10; Microbiotech/se AB, Årsta, Stockholm, Sweden) was inserted into the muscle through the standard catheter to a depth of 20 mm measured from the skin surface, ensuring that the entire probe membrane protruded beyond the plastic into the muscle. A microdialysis pump (MAB40, Microdialysis Pump Dual Chanel; Microbiotech/se AB) was connected to the probe.
The microdialysis probe was perfused at a rate of 5 µL/min with Ringer-acetate (Baxter Viaflo, Baxter Medical AB, Kista, Sweden) solution containing 0.5 mM Ringer-lactate (Baxter Viaflo, Baxter Medical AB) and 3 mM glucose (Glucose 50 mg/ml; B. Braun Melsungen AG, Melsungen, Germany) to prevent the interstitial space from draining. Three µM [14C]-lactate (specific activity: 7.4 MBq/ml; PerkinElmer Life Sciences, Boston, MA USA) was added to the Ringer-acetate solution to determine relative in vivo recovery (RR) and allow determination of interstitial concentrations of pyruvate, glutamate, lactate, and serotonin; likewise, 3 µM 3H2O (specific activity: 37 MBq/ml; PerkinElmer Life Sciences) was added to assess nutritive blood flow. The outlet tubing of the microdialysis probe was placed in a 500 µL microvial. Microdialysates were sampled every 20 min during 3 h and stored at -70°C.

**Chemical Analyses (III-IV)**

**Microdialysate**

An ISCUS Clinical Microdialysis Analyzer (Dipylon Medical AB, Solna, Sweden) analyzed concentrations of glutamate, lactate, and pyruvate. The limit of detection (LOD) for lactate was 0.1 mmol/L; for pyruvate, 10 µmol/L; and for glutamate, 1.0 µmol/L. Concentrations below 50% of LOD were reported as a concentration equaling half the LOD, while concentrations above 50% of LOD were reported as obtained.

Concentrations of muscle 5-HT were analyzed with a high-pressure liquid chromatograph with electrochemical detection; for details of this methodology, see Ghafouri et al. The LOD for 5-HT was 20 fmol/10 µL. The PAINOMICS lab at Linköping University Hospital analyzed all microdialysates.

5 µL dialysate or perfusate was pipetted into a counting vial containing 3 mL scintillation fluid (High-flash Point, Universal LSC-Cocktail, ULTIMA GOLD™, PerkinElmer, Inc.) and vortexed; β-counting was done in a liquid scintillation counter (Beckman LS 6000TA; Beckman Instruments, Inc., Fullerton, CA, USA). The RR for lactate was calculated for each sample: (cpm_p – cpm_d)/cpmp, where cpm_p was counts per min of perfusate and cpm_d was counts per min of dialysate. Interstitial levels (Ci) of the algesic substances were...
also calculated: \( (C_d - C_p)/RR + C_p \), where \( C_d \) was the concentration of substance in the dialysate, and \( C_p \) was the concentration of substance in the perfusate. Nutritive blood flow was estimated: \( 1/(cpmd/cpmp) \) for \( ^3\text{H}_2\text{O} \), where \( cpmd \) was \( ^3\text{H}_2\text{O} \) counts per min in the dialysate and \( cpmp \), in the perfusate.

**Statistical Analyses (I-IV)**

All statistical analyses were done using the Statistical Package for the Social Sciences, Windows, versions 17 and 20 (SPSS, IBM) and were performed two-tailed at a significance level of 5%.

**Descriptive statistics.** Means and standard deviations for all variables that were continuous and normally distributed. Medians and interquartile ranges for categorical variables and for continuous variables that were not normally distributed (II–IV).

**Kolmogorov-Smirnov test and Shapiro-Wilks test.** To test continuous variables for normality. If data were not normally distributed after transformation, non-parametric statistics were used (II-IV).

**Independent samples t-test.** To test for significant gender differences in age and MVCF in each session (III) and between M-TMD patients and healthy controls (IV).

**Paired samples t-test.** To test for significant between-session differences in MVCF (III).

**Mixed model analysis of variance (ANOVA) for repeated measures.** To assess, for PPTs, significant (i) main effects of time and group and (ii) interaction effects between time and group (IV).

**Two-way ANOVA for repeated measures.** With Dunnett’s post-hoc test, to analyze the significance of changes in mean values of VT, perceived intensity of vibration, perceived discomfort, VAS\(_{\text{pain}}\), VAS\(_{\text{fatigue}}\), and PPT at different contraction levels, over time, and for interaction effects between time and clenching level (II). With Bonferroni as a post-hoc test, to assess significant changes in RR over time and between sessions (III-IV) as well as to test mean values.
of pain intensity and fatigue for (i) significant main effects of time and group and (ii) interaction effects between time and group (IV).

**Kappa statistics.** To calculate inter-observer reliability of Qu-ATEBS for experimental bruxism studies (I).

**Phi coefficient.** To assess the discriminative validity of Qu-ATEBS for experimental bruxism studies (I).

**Intraclass correlation coefficient.** To calculate test-retest reliability for VT, a continuous variable (II).

**Spearman’s correlation test adjusted for multiple testing with Bonferroni correction.** To evaluate the correlation between pain intensity after experimental tooth clenching, PPT, 5-HT, glutamate, pyruvate, and lactate (III-IV).

**Friedman test.** To test for significant changes in 5-HT, glutamate, pyruvate, and lactate over time (III-IV) and for significant changes in pain intensity and fatigue and in PPTs (III). If changes were significant, the Wilcoxon signed ranks test was applied as a post-hoc test with Bonferroni correction (III-IV).

**Wilcoxon signed ranks test with Bonferroni correction.** To test for significant between-session differences at various time points for 5-HT, glutamate, pyruvate, lactate, $VAS_{\text{pain}}$, $VAS_{\text{fatigue}}$, and PPTs (III).

**Mann-Whitney U test.** To evaluate the significance of changes in 5-HT, glutamate, pyruvate, lactate, $VAS_{\text{pain}}$, $VAS_{\text{fatigue}}$, and PPTs between genders at various time points in each session (III). Also, to analyze whether differences in 5-HT, glutamate, pyruvate, lactate, $VAS_{\text{pain}}$, $VAS_{\text{fatigue}}$, duration of M-TMD, number of muscle and joints sites with pain on palpation, and characteristic pain intensity between M-TMD patients and healthy controls were significant (IV).
RESULTS

Study I
Item Generation
After reviewing the 16 selected articles, the steering group generated 52 items phrased as questions.

Face-Validity Assessment
Ten of the 11 experts invited to join the Delphi panel agreed to participate. Years of experience in orofacial pain research ranged from 10 to 35 yr, based upon publications in scientific peer-review journals. Table 5 presents the characteristics of the Delphi panel members. Figure 6 illustrates the results of the face-validity assessment.

Table 5. Characteristics of the Delphi panel members. Number of experts (N).
*Some panel members had more than one profession.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>3</td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
</tr>
<tr>
<td>Profession*</td>
<td></td>
</tr>
<tr>
<td>Psychologist</td>
<td>2</td>
</tr>
<tr>
<td>Orofacial pain specialist</td>
<td>8</td>
</tr>
<tr>
<td>Orthodontist</td>
<td>3</td>
</tr>
<tr>
<td>Orofacial pain researcher</td>
<td>10</td>
</tr>
</tbody>
</table>
Figure 6. Results of face-validity assessment in Delphi rounds I–IV, response rate, and included and excluded items.

<table>
<thead>
<tr>
<th>Round</th>
<th>Response rate</th>
<th>Included</th>
<th>↓</th>
<th>Excluded</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10/10</td>
<td>5</td>
<td></td>
<td>52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓</td>
<td></td>
<td>45 + 14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓</td>
<td></td>
<td>Undecided list</td>
</tr>
<tr>
<td>II</td>
<td>9/10</td>
<td>5 + 4</td>
<td></td>
<td>59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓</td>
<td></td>
<td>9 + 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>III</td>
<td>9/10</td>
<td>8</td>
<td>→</td>
<td>1</td>
</tr>
<tr>
<td>IV</td>
<td>9/10</td>
<td>Qu-ATEBS 8 Items</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓</td>
<td></td>
<td>Qu-ATEBS 7 Item</td>
</tr>
</tbody>
</table>

In round I, two items were excluded, 5 included, leaving 45 items on the undecided list. Fourteen new items were added to the undecided list. In round II, 48 items were excluded, 4 were included, and 7 items remained on the undecided list. In this round, the panelists suggested merging items and making them more general and less specific. The 7 items on the undecided list, along with the 9 included, were merged so that 10 items were formed. In round III, 2 items were merged and 1 excluded. The panelists suggested that each item should be a two-barreled question designed to specifically test quality of reporting and quality of design. Accordingly, the steering group rephrased each item. In round IV, 100% agreement was found for the 8-item Quality Assessment Tool for Experimental Bruxism Studies (Qu-ATEBS). The final tool contained 7 items following refinement; for details, see (I).
Assessment of Reliability and Discriminative Validity
Inter-observer reliability was acceptable ($k = 0.77$) and discriminative validity was high ($\phi$ coefficient $0.79$; $P < 0.01$).

Refinement of the Final Instrument
Item no. 8 was removed since it was not applicable to any of the reviewed studies in the reliability and discriminative validity test.

Study II
Test-Retest Reliability of VT
The intraclass correlation coefficient for test-retest reliability of VT measurements on the right masseter muscle between baseline and 10 min was good ($0.92$; 95% CI 0.81–0.96) and between baseline and 7 d, moderate ($0.59$; 95% CI 0.08–0.82). No significant time effects were seen for VT between baseline, 10 min, and 7 d ($P > 0.05$).

Vibrotactile Sensitivity
A significant increase over time was observed in VT ($P < 0.001$) with a significantly higher VT at 30, 40, 50, and 60 min compared with baseline ($P < 0.05$). No significant main effects of clenching level were observed for VT ($P > 0.05$; Figure 7).

Figure 7. Vibration threshold on the right masseter muscle in response to experimental tooth clenching. Mean ± SEM and P-values. * indicates significant difference from baseline values ($P < 0.05$).
PIV increased significantly over time ($P < 0.05$) with a significant increase at 40 min compared with baseline ($P < 0.05$, data not presented). Clenching level had no significant main effects on PIV ($P > 0.05$). No significant changes were observed over time or between contraction levels for PD (all $P$'s $> 0.05$, data not presented).

**Pressure Pain Thresholds**
A significant alteration over time was observed for PPT ($P < 0.01$) with significant decreases at 50 min and 60 min compared with baseline ($P$'s $< 0.05$). Mean PPT did not change significantly with contraction level ($P > 0.05$; Figure 8).

*Figure 8. Pressure pain threshold on the right masseter muscle in response to experimental tooth clenching. Mean ± SEM, and $P$-values. *indicates significant difference from baseline values ($P < 0.05$).*

**Intensity of Pain and Fatigue**
Clenching level and time had significant effects on mean VAS_{pain} and mean VAS_{fatigue} ($P$'s $< 0.001$). Thus, significant increases from baseline occurred in VAS_{pain} and VAS_{fatigue} at all time points between 10 and 60 min and at the 24-h follow-up ($P$'s $< 0.05$). Clenching level at 40% of MVCF increased VAS_{pain} and VAS_{fatigue} significantly compared to 10% of MVCF ($P$'s $< 0.05$). A significant interaction between contraction level and time was only found for VAS_{pain} ($P < 0.001$; Figure 9).
Study III
Subjects
There was no significant age difference between genders ($P > 0.05$). Table 2 lists characteristics of the study sample.

Maximal Voluntary Clenching Force
No significant between-session differences in MVCF (clenching: $52.7 \pm 18.6$ kg vs. control: $55.8 \pm 18.8$ kg) were observed ($P > 0.05$). Neither were there any significant differences in mean MVCF between females and males in the clenching session (females: $50.2 \pm 16$ kg vs males: $55.5 \pm 21.1$ kg; $P > 0.05$) or the control session (females: $54.1 \pm 16.7$ kg vs. males: $57.8 \pm 21.4$ kg; $P > 0.05$).

Pain Intensity and Fatigue
Table 6 presents pain intensities and perceived fatigue after the clenching and the control sessions. Pain intensity was significantly higher after the clenching task than after the control task ($P < 0.01$). No significant between-session differences in pain intensity were observed at baseline or after recovery ($P's > 0.05$). No significant between-gender differences in pain intensity occurred at any time point in either session ($P's > 0.05$).
Significantly higher levels of fatigue occurred after clenching ($P < 0.001$) and after recovery ($P < 0.01$) in the clenching session compared to the same time points in the control session. A comparison between females and males revealed no significant gender differences in the clenching session or in the control session at any time point ($P's > 0.05$).

**Pressure Pain Threshold**

Table 6 presents PPTs before and after microdialysis. No significant between-session differences in PPT occurred at any time point ($P's > 0.05$). Males had significantly higher PPTs than females at baseline and after microdialysis ($P's < 0.01$) in the clenching session. In the control session, PPT was significantly higher for males than females at baseline ($P < 0.05$) but not after microdialysis ($P > 0.05$).

**Table 6.** Median (interquartile range) pressure pain threshold (PPT, kPa), pain intensity, and intensity of fatigue (both: 0–100-mm VAS) measured at baseline, after the clenching and control tasks, and after recovery. Significant difference compared to the control session at the same time point: ** P < 0.01, and ***P < 0.001. Significant gender difference within the same session and time point: # P < 0.05 and ## P < 0.01.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After clenching / control task</th>
<th>After recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PPT</strong></td>
<td>Clenching</td>
<td>Control</td>
<td>Clenching</td>
</tr>
<tr>
<td>All</td>
<td>164 (77)</td>
<td>160 (66)</td>
<td>-</td>
</tr>
<tr>
<td>Females</td>
<td>149 (48)</td>
<td>146 (74)</td>
<td>-</td>
</tr>
<tr>
<td>Males</td>
<td>207 (111)**</td>
<td>194 (71)#</td>
<td>-</td>
</tr>
<tr>
<td><strong>Pain intensity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0 (10)</td>
<td>0 (10)</td>
<td>15 (30)*****</td>
</tr>
<tr>
<td>Females</td>
<td>0 (10)</td>
<td>0 (10)</td>
<td>25 (27.5)**</td>
</tr>
<tr>
<td>Males</td>
<td>0 (2.5)</td>
<td>0 (10)</td>
<td>10 (32.5)</td>
</tr>
<tr>
<td><strong>Fatigue</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0 (10)</td>
<td>0 (2.5)</td>
<td>40 (32.5)*****</td>
</tr>
<tr>
<td>Females</td>
<td>0 (10)</td>
<td>0 (0)</td>
<td>40 (17.5)*****</td>
</tr>
<tr>
<td>Males</td>
<td>0 (2.5)</td>
<td>0 (10)</td>
<td>45 (42.5)*****</td>
</tr>
</tbody>
</table>
Relative Recovery
Mean ± SD RR based on a 14C-lactate concentration was 29 ± 12% at baseline, 27 ± 12% after the clenching task, and 31 ± 15% after recovery in the clenching session, whereas RR in the control session was 27 ± 11% at baseline, 27 ± 12% after the control task, and 27 ± 13% after recovery. No significant differences occurred over time or between sessions (P’s > 0.05).

Serotonin
A comparison between sessions revealed no significant differences in muscle 5-HT at any time point (P’s > 0.05; Figure 10). No significant differences between females and males were observed at any time point in any session (P’s > 0.05; data not presented).

Glutamate
No significant differences occurred at any time point between sessions (P’s > 0.05; Figure 10). No gender differences were observed in the clenching or the control session at any time point (P’s > 0.05; data not presented).

Pyruvate and Lactate
Levels of interstitial muscle pyruvate did not differ at any time point between sessions (P’s > 0.05; Figure 10). No significant gender differences of muscle pyruvate were observed in any session at any time point (P’s > 0.05; data not presented).

Significant changes in interstitial levels of lactate were not detected between sessions at any time point (P’s > 0.05; Figure 10). Nor did any significant gender differences occur within sessions (P’s > 0.05; data not presented).

Blood Flow
Nutritive masseter muscle blood flow (out/inflow ratio, 3H2O) was unaltered at all time points between sessions (P’s > 0.05) and between genders in each session (P’s > 0.05; data not presented).
Correlations
After Bonferroni correction for multiple testing, no significant correlations were identified between pain intensity after experimental tooth clenching and any of the following: PPT, 5-HT, glutamate, pyruvate, or lactate.

Figure 10. Interstitial concentrations (median, 75%, 5% percentile) of 5-HT (A), glutamate (B), pyruvate (C), and lactate (D) in the masseter muscles of 30 healthy subjects in the clenching and the control sessions. No significant differences between sessions were observed at any time point.
Study IV

Subjects
There was no significant age difference between the groups \((P = 0.97)\). Table 3 lists characteristics of the study sample.

Maximal Voluntary Clenching Force
MVCF differed significantly between groups \((P < 0.05)\), and was \(42.5 \pm 20.2\) kg for the M-TMD patients and \(56.6 \pm 14.8\) kg for the controls.

Pain Intensity and Fatigue
Time \((P < 0.05)\) and group \((P < 0.001)\) had significant main effects on pain intensity, but no interaction effect was observed \((P > 0.05)\). Significant increases in pain intensity from baseline occurred after the clenching task \((P < 0.05)\) but not after recovery. Pain intensity was significantly higher for M-TMD patients compared to the control group \((P < 0.001; \text{Figure 11})\).

Time \((P < 0.001)\) and group \((P < 0.001)\) had significant main effects on fatigue. No interaction effects were detected \((P > 0.05)\). Fatigue increased significantly after the clenching task \((P < 0.001)\) and after recovery \((P < 0.05)\) compared with baseline. M-TMD patients had a significantly higher level of mean fatigue than the control group \((P < 0.001; \text{Figure 11})\).

Figure 11. Mean ± SEM pain intensity (A), and intensity of fatigue (B) measured at baseline, after the clenching task, and after recovery in 15 M-TMD patients and 15 healthy controls. Significant increase over time: #\(P < 0.05\), ###\(P < 0.001\). Significant difference between groups: ***\(P < 0.001\).
Pressure Pain Threshold
Effects of group ($P < 0.05$) and time ($P < 0.05$) on mean PPT were significant, with a significant decrease in mean PPT after microdialysis compared to baseline ($P < 0.01$). Healthy controls had a significantly higher PPT than did M-TMD patients ($P < 0.05$). No interaction effects were observed ($P > 0.05$; Figure 12).

Figure 12. Mean ± SEM pressure pain threshold (kPa) measured before baseline, and after recovery in 15 M-TMD patients and 15 healthy controls. Significant decrease over time: ##$P < 0.01$. Significant difference between groups: *$P < 0.05$.

Relative Recovery
Mean ± SD RR, based on $^{14}$C-lactate, was 20 ± 10% at baseline, 28 ± 10% after the clenching task, and 27 ± 8% after recovery for the M-TMD group, and 29 ± 11% at baseline, 28 ± 10%, and 30 ± 15% after recovery for the control group. No time effects were observed for RR; RR did not differ significantly between groups ($P$'s > 0.05).
Serotonin
The M-TMD group had a significantly higher level of interstitial 5-HT at baseline, after the clenching task, and after recovery (P’s < 0.01) than the healthy controls (Figure 13).

Glutamate
No significant differences between M-TMD patients and healthy controls were observed in interstitial levels of glutamate at any time point (P’s > 0.05; Figure 13).

Pyruvate and Lactate
Neither interstitial levels of pyruvate nor interstitial levels of lactate (all P’s > 0.05) differed significantly between groups at any time point (Figure 13).

Blood Flow
A comparison between groups revealed that the M-TMD patients had significantly lower nutritive masseter muscle blood flow (out/inflow ratio, ³H₂O) at all time points (P’s < 0.01, Figure 13).

Correlations
After Bonferroni correction for multiple testing, no significant correlations were identified at any time point in any group between (i) pain intensity, PPT, 5-HT, and glutamate; and (ii) pyruvate, lactate, fatigue, and blood flow.
Figure 13. Interstitial concentrations (median, 75% and 25% percentile) of 5-HT (A), glutamate (B), pyruvate (C), lactate (D), and out/inflow ratio ($^3$H$_2$O) in the masseter muscles of 15 M-TMD patients and 15 healthy controls at baseline, after clenching, and after recovery. *Significant difference between groups at all time points, $P < 0.01$. 
DISCUSSION

The most important findings in this thesis were that:

• Qu-ATEBS – the quality assessment tool for experimental bruxism studies – achieved face validity in the eyes of a Delphi panel of 10 experts in the field of orofacial pain, is reliable, and has high discriminative validity.

• Experimental tooth clenching is not directly related to DOMS.

• In healthy females and males and in patients with M-TMD, experimental tooth clenching is not associated with the peripheral release of 5-HT or glutamate.

• In patients with M-TMD, 5-HT is significantly higher and blood flow significantly lower compared with healthy controls.

Quality Assessment Tool for Experimental Bruxism Studies

Evidence-based medicine is defined as the “integration of the best research evidence with clinical expertise and patient values” and refers to the process whereby research is systematically sought out, reviewed, analyzed, and used for decision making in clinical practice. The overall goals of evidence-based medicine are to continually improve patient care; to implement new, effective methods with a high level of evidence; and to identify and cease use of ineffective methods.

Studies sometimes present conflicting results for a specific treatment or test. When this happens, systematic reviews play a vital role by critically analyzing all studies published on the topic and synthesizing their findings so that evidence-based conclusions can be made on the benefits or disadvantages of the treatment or test. But even if
there is a consensus in the literature, systematic reviews confirm the
quality of the studies and the reliability of the results. Systematic
reviews are therefore highly important to the concept of evidence-
based medicine.

In research that uses HEP models, it is essential that the evoked
pain mimics the clinical pain condition as closely as possible so
that pain mechanisms can be investigated and findings translated
into improved patient care. A HEP model that induces pain which
deviates from the clinical condition in pain intensity, character, and
quality might lead to conflicting results.

Due to varying standards among experimental bruxism studies and,
thus, the impossibility of comparing results, there was a need to
develop Qu-ATEBS. Use of Qu-ATEBS in a systematic review would
contribute to evidence-based literature by adding an objective, quality
assessed summary of studies’ levels of evidence and by identifying
methodological issues. Further, the optimum experimental bruxism
task that induces jaw muscle pain which mimics the features of
patients with M-TMD most closely could also be identified or
developed.

Streiner and Norman’s 5-step method was used to develop Qu-
ATEBS\textsuperscript{88}. The Delphi method was used in face-validity assessment
(step 3). This method was successfully used previously\textsuperscript{99-101}. The
most important step in the Delphi procedure is panelist selection
because the degree of heterogeneity and expertise of the Delphi
panel correlates directly with the generated results\textsuperscript{102,103}. Further, the
number of Delphi rounds must be limited; too many rounds might
incur panelist fatigue and thus compromise results\textsuperscript{89}. These factors,
together with the consensus reached in round 4 that appears to be
credible and exhibit high face validity, indicate that the Delphi panel’s
degree of expertise and heterogeneity was most likely sufficient. This
was further confirmed in the reliability and discriminative validity
assessments of Qu-ATEBS.

Qu-ATEBS exhibits high inter-observer reliability and discriminative
validity. The tool covers these areas: study aim, study sample, control
condition or group, study design, experimental bruxism task,
statistics, and interpretation of results. Each of these seven items was designed as a two-barreled question: quality of reporting and quality of design. In the following, the quality of design dimension of each of the tool's seven items will be used to assess the other three studies in this thesis.

Were the aims or hypotheses based on relevant theory?
The aims of (II–IV) were clearly described and based on relevant theory. Proprioceptive allodynia was assessed in (II), since this is one of the features of DOMS. It has been suggested that bruxism is associated with DOMS, and since tooth clenching is a risk factor for M-TMD, proprioceptive allodynia was assessed in (II). In (III) and (IV), the effect of experimental tooth clenching on the peripheral release of muscle 5HT and glutamate was investigated in healthy subjects and in patients with M-TMD. Levels of muscle 5-HT and glutamate are significantly higher in patients with M-TMD, and it was investigated whether tooth clenching can provoke a release of 5-HT and glutamate.

Were the eligibility criteria appropriate for the objectives of the studies?
Since we aimed to investigate certain aspects of pain mechanisms that might be involved in the generation of M-TMD, it was necessary to first investigate healthy subjects (II–III), which is in line with other experimental bruxism studies. In (IV) we aimed to assess the release of 5-HT and glutamate in patients with M-TMD. Pain duration in our M-TMD patients (59 mo) was lower than what other studies have reported (68.4 mo in Sweden, and 99.6 mo in the US). The pain intensity reported by our M-TMD patients (20 on a 100-mm VAS) was also lower than is reported for M-TMD patients in the literature (around 40 on a 100-mm VAS). Similar findings were observed for PPT, with our M-TMD patients having slightly higher PPTs than other M-TMD patients. The mean age of our M-TMD patients was slightly lower than reported in other studies. It seems that our M-TMD patients do not represent the typical M-TMD patient. It has been observed that TMD is comorbid with other conditions such as chronic fatigue syndrome, fibromyalgia, and irritable bowel syndrome, and that chronic
pain in general is comorbid with depression\textsuperscript{110,111}. Since no co-morbid conditions were accepted in (IV), more severe cases were excluded, which might have affected mean age and mean pain intensity of the patients. But it must be emphasized that our M-TMD patients most likely are generalizable for uncomplicated M-TMD cases. Further, our M-TMD patients had significantly lower bite forces and PPTs than the healthy controls, which is in line with the results of others\textsuperscript{106,107,112}. The eligibility criteria for the M-TMD patients, however, appear to be relevant to the objectives of (IV). If patients were on medication that would influence pain perception or suffered from another chronic pain condition or from depression diagnosed by a physician, results would most likely have been biased.

**Were the control group, control condition, or experimental condition appropriate for the studies?**

In (II), three levels of contraction (10\%, 20\%, and 40\% of MVCF) were randomly assigned to the healthy participants. These experimental conditions were appropriate for investigating proprioceptive alldynia after experimental tooth clenching exercises, but a control condition without a clenching task would have improved the study. The vibration threshold in (II) increased significantly over time after experimental tooth clenching. One study has observed that tactile detection thresholds increase over time without a tooth clenching task\textsuperscript{113}. In (III), healthy subjects were randomly allocated to a repetitive experimental tooth clenching task in one session and a control task in the other session. In (IV), which consisted of one session, the M-TMD patients and the control group performed a repetitive experimental tooth clenching task. Thus, the control condition in (III) and control group in (IV) were appropriately selected for the objectives of these studies; however, a control condition without a clenching task would have improved the results of (II).

**Was the study design appropriately selected for the objectives of the studies?**

To investigate the occurrence of proprioceptive alldynia after experimental tooth clenching, an intense vibratory stimulus was applied to the masseter muscle after each bout of clenching and at
the 24- and 48-h follow-ups. Other variables measured in (II) to investigate the relationship between experimental tooth clenching and DOMS were pain intensity, fatigue, and PPT. Since no operationalized criteria exist for DOMS, all potentially characteristic variables were assessed to reach the most reliable conclusions possible. VT and PPT measurements on the masseter muscle have acceptable reliability\(^91,114\).

In (III) and (IV) microdialysis was used to investigate the release of 5HT, glutamate, pyruvate, and lactate after experimental tooth clenching exercises. This methodology was previously used to study these substances in healthy subjects and patients with trapezius myalgia after low-force muscle exercise\(^81,82,115\). Other variables assessed in (III) and (IV) were pain intensity, fatigue, and PPT, as previously described.

Thus, the designs of the studies seem appropriate for the objectives in (II), (III), and (IV), and are sufficiently described so that replication is possible.

Were the experimental bruxism tasks appropriately selected for the objectives of this study?

The main goal of using experimental bruxism tasks is to increase knowledge of pain mechanisms and to establish the role of bruxism in the development or maintenance of TMD without any of the confounding factors that tend to be observed in clinical pain\(^38\). One of the benefits of using experimental bruxism models is that pain mechanisms can be investigated under isolated and controlled settings without any confounding factors\(^38\). It is therefore vital that the pain evoked by an experimental bruxism task mimics the features of M-TMD observed in patients in general, and thus, produces a pain intensity of approximately 40 (on a 100-mm VAS)\(^9,105\) and a significantly lower PPT\(^106,107\) and bite force\(^112\) than the control condition or in the healthy control group. Several experimental bruxism models have been developed. Some studies have used maximal voluntary clenching\(^54,55,57,116\), while others have used clenching at submaximal levels of MVC\(^46,50,117\). Studies that use maximal clenching provoke considerably higher pain intensities
compared to what is observed in M-TMD patients. It seems that these high-intensity clenching tasks do not reflect M-TMD patients in general, and their validity is therefore questionable. Furthermore, no data so far suggest that TMD patients engage in tooth clenching at maximal bite force. So studies with such methodology might present results that are not representative for M-TMD patients. It has been suggested that patients with TMD perform tooth clenching for longer periods\textsuperscript{60}, so it is unlikely that tooth clenching occurs at maximal bite force since maximal bite force can only be maintained for a short period of time\textsuperscript{61}. Thus, an experimental tooth clenching task consisting of continuous isometric contractions at a submaximal level of MVCF might be more suitable and valid for reproducing the pain characteristics of M-TMD patients in healthy subjects. A low-level clenching task can produce pain and symptoms in otherwise healthy subjects, which might lead to a diagnosis of M-TMD per the RDC-TMD\textsuperscript{4}, following the clenching task\textsuperscript{118-120}. Prolonged periods of tooth clenching such as experimental tooth clenching until exhaustion\textsuperscript{46} might be more representative of TMD patients.

Low levels of continuous clenching at 10% of MVCF for 30 min provoked low levels of pain with a mean value of approximately 13 on a 100-mm VAS\textsuperscript{47}. Similar results were observed in another study after a repetitious experimental tooth clenching task at 50% of MVCF where each bout lasted for 30 sec and was repeated every 1.5 min for 30 min\textsuperscript{48}. A sustained experimental tooth clenching task at 10% of MVCF for 60 min was associated with slightly higher pain intensity, around 28 on a 100-mm VAS\textsuperscript{50}. In that study, mechanical hyperalgesia was not developed, but bite force was significantly reduced immediately after the clenching task\textsuperscript{50}. A continuous clenching task for 15 min at 25% of MVCF evoked moderate levels of pain in healthy subjects, but no mechanical hyperalgesia developed\textsuperscript{53}. One study\textsuperscript{46} observed that clenching until exhaustion was associated with low levels of pain (around 15 on a 100-mm VAS) and mechanical hyperalgesia immediately after the clenching task at 7.5% of MVCF and one day after. Slightly higher levels of pain were observed after clenching at 40% of MVCF, but no mechanical hyperalgesia developed\textsuperscript{46}. The study reported that endurance for clenching at 7.5% of MVCF was 157 min and at 40%, 1.4 min\textsuperscript{46}. 

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In (II), the subjects conducted experimental tooth clenching at 10%, 20%, and 40% of MVCF. Six bouts of clenching, each bout lasting 5 min, were performed for each contraction level. Experimental tooth clenching was associated with moderate levels of pain that persisted one day after the clenching task and short-term mechanical hyperalgesia, which resembles the characteristics of M-TMD patients\textsuperscript{105,106}. Unfortunately, bite force was not assessed immediately after the clenching task or at the follow-ups. A possible explanation for the conflicting results compared to the study by Farella et al.\textsuperscript{46} is most likely methodological differences. In the Farella et al. study\textsuperscript{46}, the subjects performed clenching exercises at different contraction levels until exhaustion, while in our study the subjects performed the clenching task repetitively during 60 min. The experimental tooth clenching model used in (II) seems to be valid due to the pain characteristics that the healthy subjects developed. It seems that development of mechanical hyperalgesia is related to level of contraction or duration of the experimental tooth clenching task.

In (III) and (IV) another experimental tooth clenching model was used, i.e., clenching 20 times at a contraction level of 50% of MVCF for 30 s, with 30-s resting intervals between each bout of clenching. This model was chosen because it had previously been used with microdialysis in the masseter muscle and because this model evokes low levels of pain\textsuperscript{48}, similar to baseline values reported by the M-TMD patients. This experimental tooth clenching model was used in (III) and (IV) and was associated with the development of mechanical hyperalgesia after the clenching task in (IV).

Thus, it seems that the experimental tooth clenching models used in (II–IV) were valid, mimicked our M-TMD patients’ pain characteristics accurately, and were appropriately selected for their objectives. Although these experimental tooth clenching models might not exhibit high external validity regarding the clenching task per se, a clenching task at a lower intensity for a longer period of time might have been equally as good. But because the trials in (III) and (IV) were time consuming, a prolonged clenching task at a lower intensity would have been impractical.
Were the statistical methods and data appropriate for the objectives of the studies?
In all studies, descriptive statistics with mean values and standard deviations were calculated for all variables that were normally distributed. Medians and interquartile ranges were calculated for categorical variables and for continuous variables that were not normally distributed. Continuous variables were tested for normal distribution. When appropriate, parametric and non-parametric statistics were used to test for significant alterations over time, between groups, and between genders. The statistical analyses used seem to be appropriate for the objectives of the variables in the studies; for details see the Statistical Analyses section.

Were aims and hypotheses clearly addressed in the conclusions and relevant to the objectives?
The conclusions of the studies summarize the results, and each of the hypotheses was addressed in the conclusions, except in (I), which had no hypothesis.

**Association Between Experimental Tooth Clenching and Delayed Onset Muscle Soreness**
The results indicated that experimental tooth clenching at different contraction levels evoked pain, fatigue, and short lasting mechanical hyperalgesia, but no proprioceptive allodynia was detectable. Thus, it seems that experimental tooth clenching at different contraction levels is not directly related to DOMS. The most likely explanation for the absence of DOMS might be ascribed to the experimental bruxism model that was used, which consisted of concentric contractions. The vast majority of studies suggest that DOMS is mainly associated with eccentric contractions\(^{26,121,122}\). One study, however, demonstrated an association between concentric contractions and DOMS in limb muscles\(^{27}\).

Another interesting finding in (II) was that VT increased significantly over time but was unrelated to the level of contraction. Previous studies have observed that TMD pain patients exhibit a higher VT than healthy controls\(^{123,124}\). The most likely interpretation of our results is that tooth clenching was not the cause of the increased
VT, instead it was suggested that vibrotactile adaptation impairs the sense of vibration. It has been observed that the tactile detection threshold increases significantly after experimental clenching exercises. The same study confirmed these findings in a repetition of the trial without tooth clenching. The group concluded that the modulated tactile detection threshold was a result of habituation. Other research groups have shown that vibratory stimulus can desensitize cutaneous mechanoreceptive afferents, causing higher VTs. This agrees with our results, which indicate that increased VTs are due to an adaptation effect.

Thus, it seems that concentric contractions of the masticatory muscles are not directly associated with DOMS or altered vibrotactile sensitivity. It is possible that an experimental bruxism model with eccentric contractions would have yielded different results.

**Algesic Substances, Pain, and Mechanical Hyperalgesia after Experimental Tooth Clenching**

Experimental tooth clenching is associated with development of relative ischemia, and the ischemic condition can provoke a release of algesic substances and sensitize and activate muscle nociceptors.

The level of 5-HT was not significantly altered after experimental tooth clenching in healthy subjects or in patients with M-TMD, which is consistent with a previous finding in patients with trapezius myalgia. It cannot be excluded that another experimental tooth clenching task, i.e., clenching until exhaustion, would have provoked a release of 5-HT. The level of 5-HT was significantly higher in patients with M-TMD compared to healthy controls, which agrees with the results of others. 5-HT might not have an initiating role in M-TMD, instead it might have a perpetuating role in chronic muscle pain by increasing the effects of other algesic substances. Furthermore, previous studies demonstrated a significant correlation between the level of 5-HT, pain intensity, and PPT. One explanation for the absence of a significant correlation in our studies might be ascribed to the characteristics of the M-TMD patients; our patients had lower mean pain intensities compared with the patients in previous studies.
The level of muscle glutamate was not increased in response to experimental tooth clenching for healthy controls or patients with M-TMD. Another study, however, found significantly increased levels of glutamate after low-force muscle exercise in both healthy controls and patients with trapezius myalgia\(^81\). Our contradictory results could possibly be ascribed to the clenching task. It cannot be excluded that the jaw muscles recovered during the relaxation period, hence no increase of glutamate. In the study by Rosendal et al., the patients performed muscle exercise continuously for 20 min without any relaxation\(^81\). A significant difference in glutamate levels between M-TMD patients and healthy controls was not observed. Other studies have reported that patients with trapezius myalgia and M-TMD have significantly higher levels of glutamate than healthy subjects\(^80,81,95\). Although glutamate levels in our M-TMD patients were similar to those reported by Castrillon et al.\(^80\), baseline glutamate levels in our healthy controls were higher than in their controls, even though our healthy subjects reported no pain at baseline. This could explain why we observed no significant difference between our M-TMD patients and healthy controls. Differences in perfusion rate and the method used to assess glutamate concentration in the dialysates might explain the higher glutamate levels in our healthy subjects. Furthermore, after insertion of the microdialysis probe, the stabilization period, designed to allow the tissue to recover from possible changes in the interstitial environment, was 120 min in (III) and (IV). The Castrillon et al. study\(^80\) used a 40-min stabilization period. Flodgren et al.\(^128\), observed that a longer stabilization period is needed for glutamate to stabilize in patients with muscle pain (150 min), while a 120-min stabilization period is sufficient in healthy controls. Thus, differences in the stabilization period could also explain these divergent results.

According to our results, the pain intensity and the mechanical hyperalgesia that developed after experimental tooth clenching were not caused by release of 5-HT or glutamate. It might be that 5-HT has a more habitual role in chronic muscle pain\(^82\). It cannot be excluded that other substances that were not investigated in (III) and (IV) were released in association with the experimental tooth clenching task and activated the muscle nociceptors. Such substances include
ATP\textsuperscript{129}, K\textsuperscript{+}, and H\textsuperscript{+} ions\textsuperscript{130,131}; prostaglandins\textsuperscript{132, 133}; cytokines\textsuperscript{134,135}; and neuropeptides such as bradykinin\textsuperscript{136} and calcitonin gene-related peptide\textsuperscript{137}. Our study did not investigate these substances, and further research would be required to establish such a relationship and its role in chronic pain.

Muscle exercise and ischemia are associated with a reduction in pH, and the level of H\textsuperscript{+} is increased\textsuperscript{127}. It has been observed that H\textsuperscript{+} can activate ASIC and TRPV1 receptors and that the density of these receptors are increased in response to reduced pH\textsuperscript{62}, thereby causing pain. Experimental pain studies have observed that intramuscular injection with a low pH solution is associated with pain and mechanical hyperalgesia\textsuperscript{138,139}. The healthy subjects in (III) and (IV) perceived low levels of pain, which might be ascribed to a reduced pH.

Another candidate for causing pain after experimental tooth clenching exercises is ATP, which exists in all cells of the body and is involved in muscle contractions. During muscle exercise, levels of extracellular muscle ATP increase considerably\textsuperscript{140,141}, as they do in response to muscle trauma\textsuperscript{142}. ATP targets and activates receptor molecules in the membranes of the nociceptive endings; P2X3 receptors\textsuperscript{129}. Thus, the increased levels of extracellular muscle ATP might activate P2X3 receptors, thereby causing pain. Further, intramuscular injections in the trapezius muscle with ATP provokes pain\textsuperscript{143}.

After the experimental tooth clenching task, the M-TMD patients developed significantly higher levels of pain compared to the healthy controls. A likely explanation for this might be the significantly higher levels of muscle 5-HT that were observed in the M-TMD patients. 5-HT targets 5-HT\textsubscript{3} receptors in the membranes of nociceptive endings. Activation of that receptor leads to the formation of protein kinase A through cyclic adenosine monophosphate. Protein kinase A heightens the sensitivity of the TTX-r sodium channels\textsuperscript{67}, the primary afferents are shifted into a hyperpolarized state, and the activation threshold is lowered\textsuperscript{63}. The density of TTX-r sodium channels is increased when a nociceptive ending is sensitized due to increased
 Intramuscular injection with 5-HT provokes pain and hyperalgesia in healthy subjects. The significantly higher levels of 5-HT might have contributed to the significantly lower PPTs observed in the patients with M-TMD compared to the healthy subjects. Glutamate injections in a healthy masseter muscle lead to mechanical hyperalgesia. After the clenching task, the PPTs of the M-TMD patients were further reduced. This reduction in PPT is most likely not caused by 5-HT or glutamate since these levels were unaltered over time. The reduction in PPT might be ascribed to the release of other algesic substances that were not investigated in the present study, as previously discussed. Furthermore, it is important to emphasize that conflicting evidence exists for the association between experimental tooth clenching and mechanical hyperalgesia in healthy subjects. Methodological differences in the clenching tasks most likely explain the divergent results.

**Experimental Tooth Clenching and Blood Flow**

Our results indicate that blood flow was not altered in association with the experimental tooth clenching model in the healthy subjects (III) or in the patients with M-TMD (IV). The study by Monteiro and Kopp used a clenching level of 50% of MVCF and instructed participants to clench as long as possible; relative ischemia developed after experimental tooth clenching. Our tooth clenching model consisted of 20 bouts of clenching at 50% of MVCF with 30 s of relaxation between each bout of clenching. The jaw muscles and blood flow most likely recovered during the relaxation periods.

Blood flow in the patients with M-TMD was significantly lower compared to the healthy subjects throughout the trial, which
agrees with another study that investigated blood flow in patients with trapezius myalgia\textsuperscript{42}. Patients with trapezius myalgia have significantly higher muscle tension during rest between muscle contractions compared to healthy controls\textsuperscript{146}. Psychological stress and anxiety, as well as sustained or repetitive contractions, are associated with increased muscle tension\textsuperscript{147}. It has been observed that higher muscle tension might increase intramuscular pressure with an alteration in blood flow, thus possibly provoking venous congestion and hypoxia\textsuperscript{42}. Further, a muscle lesion can give rise to a muscle contracture, i.e., contraction not elicited by electrical nerve activity. Sarcoplasmatic reticulum in the muscle fibers can rupture in response to the muscle lesion, and calcium is released. Calcium is needed for the actin and myosin filaments to slide along each other, and a contracture might be generated. This process requires a high supply of oxygen, which might cause hypoxia. It is also energy dependent with subsequent depletion of ATP, which may cause failure of the calcium pump. If the calcium pump is impaired, calcium levels cannot be restored and the contracture, as well as the hypoxia, persists\textsuperscript{15}. As a result of the muscle lesion, vasoactive substances may be released, causing an edema, which participates in the venous congestion and contributes to the hypoxia\textsuperscript{15}. One could expect development of hypoxia to be accompanied by increased levels of muscle pyruvate and lactate in the patients with M-TMD. But it has been observed that patients with trapezius myalgia do not exhibit altered levels of pyruvate or lactate, compared to healthy subjects\textsuperscript{148}. Taken together, several factors might contribute to the impaired blood flow observed in M-TMD patients.

Reduced blood flow might indicate hypoxia and might therefore be responsible for the significantly higher levels of 5-HT observed in patients with M-TMD since an ischemic condition can provoke 5-HT release\textsuperscript{15}.

**Experimental Tooth Clenching and Metabolism**

In (II–IV), participants performed repetitive experimental tooth clenching tasks. To perform muscle contractions, energy is required in the form of ATP, which is degraded into adenosine diphosphate (ADP) during contraction. But skeletal muscles only contain ATP
sufficient for a few seconds of muscle contraction. However, other processes that occur within the muscle fibers can recover ATP by conversion of ADP into ATP: glycolysis and oxidative metabolism. The re-converted ATP can be used to prolong muscle contraction\textsuperscript{149}. During glycolysis, which is an anaerobic process, glucose can be metabolized into pyruvate and ATP. The pyruvate molecules can be further metabolized into lactate in an anaerobic process. The energy produced in glycolysis can be used for short, intense bouts of muscle exercise such as the experimental tooth clenching tasks. Oxidative metabolism is an aerobic process and occurs in the mitochondria of the muscle fibers. During this process, ATP is created and this energy is mainly used for muscle exercise that continues for several hours\textsuperscript{150,151}.

Experimental tooth clenching did not significantly alter levels of interstitial pyruvate in patients with M-TMD (IV) or healthy controls (III-IV). Studies have demonstrated that levels of interstitial muscle pyruvate and lactate are increased in patients with fibromyalgia and chronic work-related trapezius myalgia compared with healthy subjects\textsuperscript{115,152}. Flodgren et al\textsuperscript{153} observed that both muscle pyruvate and lactate increased in healthy females after low-force muscle exercise, which no other study has yet corroborated\textsuperscript{115}.

It has been argued that high levels of muscle pyruvate indicate reduced tissue oxygenation\textsuperscript{154}, in which case pyruvate can be metabolized into lactate\textsuperscript{155}, but the opposite is also possible, i.e., a conversion of lactate into pyruvate by lactate dehydrogenase\textsuperscript{115}. The role of lactate in muscle exercise is highly complex, but it has been suggested that lactate could be an indicator of anaerobic metabolism and tissue hypoxia\textsuperscript{95,153,156}.

It is possible that a different tooth clenching model, such as clenching until exhaustion, would have yielded different results.

**Microdialysis**

The release of 5-HT, glutamate, pyruvate, and lactate after the experimental tooth clenching task was determined by microdialysis in (III) and (IV). This method has been previously used to assess the biochemical environment in muscle tissue\textsuperscript{79,81,96,127,152,157,158}. 

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Ultrasonography was not used during insertion of the microdialysis probe, which has been done in other studies\textsuperscript{96,115}. One might argue whether the probe was within the masseter muscle. But the methodology we used had been used previously\textsuperscript{93}. Before the probe penetrated the muscle fascia of the masseter muscle, light resistance was felt; after this, the probe was inserted approximately 10 mm, ensuring that it had been placed within the muscle tissue.

It has been demonstrated that immediately after insertion of a microdialysis probe into muscle tissue, levels of 5-HT are increased, which is referred to as the trauma phase\textsuperscript{79}. To avoid this effect, our study design contained a stabilization period of 2 h.

Since all molecules in the interstitial space cannot be recovered in the microdialysates, dialysate concentrations do not reflect the true concentrations of the measured substances. Thus, [\textsuperscript{14}C]-lactate was added to the perfusion medium to allow determination of RR\textsuperscript{87} and interstitial concentrations of 5-HT, glutamate, pyruvate, and lactate. The molecular masses of 5HT, glutamate, pyruvate, and lactate are similar to the mass of [\textsuperscript{14}C]-lactate, which was therefore chosen to calculate RR. Others have previously used this method\textsuperscript{81,93,95,96}. Several factors have been suggested to affect RR, such as flow rate, diffusion rate, and type of membrane\textsuperscript{85}. Our results indicate that RR for [\textsuperscript{14}C]-lactate was stable over time for healthy subjects and patients with M-TMD. Thus, RR was constant and seems to be reliable.

**Strengths and Limitations**
One strength in (I) was that the methodology used to develop Qu-ATEBS – the Streiner and Norman method\textsuperscript{99,159} and the Delphi method\textsuperscript{99,101,160} – had been successfully used before. The fact that a consensus was reached in round 4 and that Qu-ATEBS has high discriminative validity reflects the Delphi panel’s heterogeneity and expertise, which is a strength. During assessment of reliability and discriminative validity, 11 studies were selected, of which 5 had also been used in item generation. This could have biased results and is a limitation. But after additional statistical analysis of the 6 unique articles, inter-observer reliability and discriminative validity improved considerably, despite the small number of articles. Another
limitation might be that intra-observer reliability was not tested. Most likely, intra-observer reliability would be high due to hang-over effects, which a post-hoc analysis confirmed.

One strength in (II) is that different contraction levels were used in the experimental tooth clenching exercises and that follow-ups were made 24 and 48 h after the clenching tasks to assess DOMS. The Vibrameter had been introduced in orofacial pain research as an alternative to the Rydel-Seiffer graded tuning fork at the time of (II), and use of the Vibrameter is a strength. The Vibrameter exhibits moderate, long-term reliability for VT measurement on the masseter muscle, however, validity is not yet known and further research is required to elucidate this before the Vibrameter can be used as a valid instrument in orofacial pain research. A control task with no clenching would have improved the study.

A strength is that an experimental tooth clenching model was used that induced levels of pain in healthy subjects similar to those reported at baseline by the M-TMD patients. One limitation of (III) and (IV) is that some of the microdialysates had no detectable levels of any of the substances, which might have affected the results.

Other methodological strengths are that a single-blinded approach was applied in (II) and (III). A randomization procedure was used to allocate the subjects to a tooth clenching task (II) or a control condition (III). A control task was used in (III) to investigate intramuscular events after experimental tooth clenching while a control group was used in (IV). In addition, a researcher blinded to participants’ group assignment conducted the biochemical analyses. The study samples in (II–IV) were small, but each study sample was based on a power analysis.

One limitation in (II–IV) is that we did not control the menstrual cycle phases in the females. Sex hormone levels affect pain levels\textsuperscript{161}, but the magnitude of this effect is probably low\textsuperscript{162,163}. The women in our studies were most likely in different phases of the cycle, which would negate any effects.
CONCLUSIONS

Study I
- Qu-ATEBS – the 7-item, evidence-based quality assessment tool for use in systematic reviews of experimental bruxism studies, exhibits face validity, good inter-observer reliability and excellent discriminative validity.

Study II
In healthy subjects:
- The vibration threshold increased over time in response to experimental tooth clenching, most likely due to vibrotactile adaptation.
- Pain was not evoked in response to intense vibratory stimulus after experimental tooth clenching, indicating lack of proprioceptive allodynia.
- Moderate levels of pain and fatigue and short-lasting mechanical hyperalgesia developed over time after experimental tooth clenching.

Study III
In healthy subjects:
- Levels of 5-HT, glutamate, pyruvate and lactate were not increased in response to experimental tooth clenching.
- Females did not have significantly higher levels of 5-HT, glutamate, pyruvate, or lactate than males in response to experimental tooth clenching.
Study IV

- M-TMD patients had significantly higher levels of 5-HT than healthy controls at rest, and no significant differences between groups were identified for glutamate, pyruvate, or lactate.
- The levels of these substances were not significantly altered in response to clenching exercises in M-TMD patients and healthy controls.
- 5-HT and glutamate did not correlate significantly with pain intensity or PPT in M-TMD patients and healthy controls.
- Experimental tooth clenching was associated with low to moderate levels of pain and fatigue, and mechanical hyperalgesia in M-TMD patients and healthy subjects. Pain intensity and fatigue were significantly higher in M-TMD patients than in healthy controls. PPT was significantly lower in M-TMD patients compared to healthy controls.

Clinical Implications and Future Aspects

The results of this thesis do not have immediate clinical implications per se, but they are necessary steps in broadening our understanding of the pain mechanisms that are related to bruxism. Only then may findings be used in a clinical context to improve diagnostic procedures and enhance treatment strategies. Several approaches, based on the results, can be used to further study pain mechanisms of M-TMD. First, use of the Qu-ATEBS should help improve comparability between experimental bruxism studies. Second, manifestation of DOMS after other types of bruxism should be investigated. Third, release of other algesic substances in relation to bruxism should be assessed.
In the 2005 fall semester as an undergraduate dental student, I came into contact with a professor at the Department of Orofacial Pain and Jaw Function. I was planning my degree project and wanted to write about pain. So this professor tossed me a tantalizing topic: “Comparison of pain thresholds and pain tolerance levels between Middle Easterners and between genders”. He shared his enthusiasm and joy for research, it was contagious and it inspired me – my degree project was the beginning of a journey into orofacial pain research. For this, and for your excellent support, dedication, and guidance, I will be forever grateful, Professor Thomas List!

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REFERENCES


100. Lambe P, Bristow D. What are the most important non-academic attributes of good doctors? A Delphi survey of clinicians. *Med Teach* 2010; 32: e347-54.


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EXPERIMENTAL TOOTH CLENCHING

A model for studying mechanisms of muscle pain