Interstitial Glutamate Concentration is Elevated in the Masseter Muscle of Myofascial Temporomandibular Disorder Patients

Eduardo E. Castrillon, DDS, MSc, PhD
Postdoctoral Fellow
Department of Clinical Oral Physiology
School of Dentistry
University of Aarhus, Aarhus, Denmark

Malin Ernberg, DDS, PhD
Associate Professor
Department of Clinical Oral Physiology
Institute of Odontology
Kariolinska Institutet, Huddinge, Sweden

Brian E. Cairns, PhD, RPh
Associate Professor and
Canada Research Chair in
Neuropharmacology
Faculty of Pharmaceutical Sciences
The University of British Columbia
Vancouver, British Columbia, Canada

Kelun Wang, DDS, PhD
Associate Professor
Orofacial Pain Laboratory
Center for Sensory-Motor Interaction
Aalborg University, Aalborg, Denmark
Department of Oral and Maxillofacial
Surgery
Aalborg Hospital, Aalborg, Denmark

Barry J. Sessle, BDS, MDS, BSc, PhD, DSc (hc)
Professor and Canada Research Chair
Faculty of Dentistry
The University of Toronto
Toronto, Ontario, Canada

Lars Arendt-Nielsen, MScEE, PhD, Dr Med
Professor
Center for Sensory-Motor Interaction
Aalborg University, Aalborg, Denmark

Peter Svensson, DDS, PhD, Dr Odont
Professor and Chairman
Department of Clinical Oral Physiology
School of Dentistry
University of Aarhus, Aarhus, Denmark

Correspondence to:
Dr Eduardo Castrillon
Department of Clinical Oral Physiology
School of Dentistry
University of Aarhus
Vennelyst Boulevard 9
DK-8000 Aarhus C, Denmark
Fax: +45 86195665
Email: ecastrillon@odont.au.dk

Aim: *To determine if myofascial temporomandibular disorder (TMD) pain patients have elevated interstitial concentrations of glutamate in the masseter muscle.* ***Methods:*** *Thirteen patients (3 men, 10 women) diagnosed with myofascial TMD pain and 10 (2 men, 8 women) age-matched healthy controls participated in a single microdialysis session. Microdialysis was performed in the patients in the most painful point of the masseter muscle, while in the healthy subjects a standardized point in the muscle was chosen. Two microdialysis samples were collected over 40-minute epochs. A blood sample was also taken for analysis of plasma glutamate concentration. Numeric rating scale (NRS) scores of pain intensity and unpleasantness, McGill Pain Questionnaire data, pain drawing areas, pressure pain thresholds, pressure pain tolerances, maximum voluntary bite force, and maximum voluntary mouth opening were collected as secondary measurements.* ***Results:*** *The median concentration of glutamate in the masseter muscle of the myofascial TMD pain patients (7.5 ± 2.6 μM) was significantly higher (*$P < .023$*, Mann-Whitney test) than the concentration in healthy controls (0.5 ± 0.4 μM). There were, however, no significant correlations between glutamate concentrations in the masseter muscle and NRS pain scores. Plasma concentrations of glutamate were similar in patients and healthy controls.* ***Conclusions:*** *The present study demonstrates a marked increase in interstitial glutamate concentration in the masseter muscle of myofascial TMD pain patients. These novel findings suggest that peripheral glutamate could be involved in the pathophysiology of myofascial TMD pain.* J OROFACIAL PAIN 2010;24:350–360

Key words: glutamate, masseter muscle, microdialysis, orofacial pain, temporomandibular disorders

Patients with a myofascial temporomandibular disorder (TMD) characteristically have symptoms of localized ongoing and activity-provoked masticatory muscle pain that occurs in the absence of demonstrable muscle pathology.[1–4] The localized nature of muscle pain in myofascial TMD suggests that a peripheral mechanistic component may contribute to these disorders, although it has been difficult to demonstrate significant localized changes in the muscle tissue associated with this pain. One exception has been the finding that the concentration of the biogenic amine serotonin in the human masseter muscle appears to be elevated in myofascial TMD patients and is positively correlated with pain and inversely correlated with pressure pain threshold (PPT) and tolerance (PPTOL).[5] More recently, it has been reported that concentrations of the excitatory amino acid glutamate may also be elevated in chronic myalgia of the trapezius muscle and that there is an association between glutamate tissue concentrations and pain as well as mechanical sensitivity in

Table 1 Clinical Characteristics at Baseline, Mean Values (SEM) (RDC/TMD Findings)

	TMD pain patients (n = 13)	Healthy controls (n = 10)	*P* value	Test
Age (years)	28.7 (2.0)	27.1 (1.6)	.476	Mann-Whitney
Duration of myofascial TMD pain (years)	8.2 (1.7)	0.0 (0.0)	< .001*	Mann-Whitney
Maximum unassisted opening without pain (mm)	47.8 (2.5)	51.9 (1.9)	.239	Mann-Whitney
Maximum unassisted opening pain included (mm)	51.2 (2.7)	54.4 (2.0)	.385	Mann-Whitney
Maximum assisted opening pain included (mm)	52.2 (2.9)	55.7 (2.1)	.363	*t* test
No. of muscle sites with pain on palpation (0 to 20)	9.5 (1.2)	0.5 (0.3)	< .001*	Mann-Whitney
No. of temporomandibular joint (TMJ) sites with pain on palpation (0 to 4)	1.1 (0.3)	0.0 (0.0)		
No. of patients with TMJ sounds	3	1		

this condition.[6] At present, it is unclear whether a similar increase in interstitial glutamate concentration also occurs in the masseter muscle of myofascial TMD patients.

It has been shown that small volume (0.2 mL) injections of a high concentration (500 to 1000 mM) of glutamate into the human masseter muscle reliably evoke localized muscle pain and induce localized mechanical sensitization, symptoms similar to those reported by myofascial TMD patients.[7–14] In humans, muscle pain and mechanical sensitivity associated with the artificial elevation of masseter muscle glutamate concentration appear to be mediated by activation of peripheral N-methyl-d-aspartate (NMDA) receptors, although other receptor mechanisms likely also contribute to these effects of glutamate.[9,10,15] Animal studies have provided evidence that NMDA excites slowly conducting, putative nociceptive masseter afferent fibers and that almost half of the trigeminal ganglion neurons that innervate the masseter muscle express NMDA receptors.[16] These animal studies have also recently demonstrated that a two to three times increase in glutamate concentration in the masseter muscle is sufficient to excite and mechanically sensitize masseter afferent fibers through activation of these peripheral NMDA receptors.[10] These findings imply that relatively small increases in masseter muscle glutamate levels may be sufficient to alter pain perception.

As a result of these various findings, we speculated that elevated levels of interstitial glutamate in craniofacial muscles may play a role in the development and maintenance of myofascial TMD pain. The aim of the present study was to investigate if myofascial TMD patients have elevated interstitial glutamate concentrations in the masseter muscle. To accomplish this, we employed intramuscular microdialysis, a technique that has been widely used in human skeletal muscle,[5,6,17–20] to estimate interstitial glutamate concentrations in the masseter muscle of chronic myofascial TMD patients and healthy controls.

Materials and Methods

Patients and Healthy Controls

The study involved 13 patients (3 men, 10 women) (Table 1) who were recruited from consecutive patients referred to the Department of Clinical Oral Physiology, School of Dentistry, University of Aarhus, Denmark, where the study took place. The study was approved by the local Ethics Committee and conducted in accordance with the Helsinki Declaration. All the patients and the healthy controls (see below) read and signed informed consent forms. Inclusion criteria were a diagnosis of myofascial TMD pain 1a or 1b.[21] Patients were required to have ongoing pain and pain on palpation at a minimum of one site in the masseter muscle region with a characteristic pain intensity of more than 2 out of 10 on a visual analog scale (VAS) for at least 2 months prior to the experiment. Exclusion criteria were pregnancy; presence of systemic musculoskeletal pain disorders, such as fibromyalgia or inflammatory joint disease, eg, rheumatoid arthritis[22]; serious systemic diseases, including current malignancies; and chronic administration of psychiatric, analgesic, or other medications that might influence their response to pain.

Ten healthy subjects participated as a control group (two men, eight women) and were selected to be similar to the cases except for the pain condition. They had no pain from the masticatory muscles, no history of TMD problems, and were matched to the TMD

patients with respect to age, gender, and use of contraceptives. The baseline clinical characteristics for the patients and healthy controls are shown in Table 1.

Experimental Protocol

A clinical examination was performed to confirm a diagnosis of myofascial TMD according to the Research Diagnostic Criteria (RDC) for TMD.[21] All patients and healthy controls participated in a single microdialysis session, in which a blood sample was also taken for analysis of the plasma glutamate level prior to the experiment. Figure 1 illustrates the experimental protocol followed.

Microdialysis

Skin surface anesthesia was obtained by application of a local anesthetic cream (EMLA; Lidocaine 25 mg/g, Prilocaine 25 mg/g, AstraZeneca) to the skin overlying the masseter muscle for at least 20 minutes before inserting the microdialysis probe. EMLA provides skin surface anesthesia without penetrating the muscle.[23] A standard catheter (1.2 mm diameter, Venflon 2, Boc Ohmeda) was inserted into the masseter muscle through the skin at an angle of 45° to a depth of 20 mm. The catheter needle was then withdrawn and the catheter pulled out 10 mm. Finally, the catheter was cut 10 mm from the skin surface.[5] In patients with myofascial TMD pain, the most painful point in the masseter muscle determined by manual palpation corresponded to the insertion site of the probe, while in the healthy subjects a standardized point midway between the upper and lower border of the masseter muscle and 1 cm posterior to its anterior border was chosen.[5] A microdialysis probe (MAB 3, Microbiotech) with a membrane length of 10 mm, a diameter of 0.5 mm, and a shaft length of 20 mm was inserted into the masseter muscle through the catheter and protruded 10 mm outside the catheter. The probe was connected to a microinfusion pump (CMA/102, Carnegie Medicine) that perfused the dialysis probe at 2 μL/minutes with sterile phosphate buffered-isotonic saline (pH = 7.4)[5] for a total of 80 minutes. Two samples of 80 μL were collected; the first during a 40-minute stabilization period and the second during the subsequent 40-minute collection. The subject was seated comfortably in a dental chair throughout the procedure.

Glutamate Concentration

Dialysate and plasma glutamate concentrations were analyzed at the Clinical Research Department, Department of Dental Medicine, Karolinska Institutet, Huddinge, Sweden, with a commercial enzyme-based assay that measures L-glutamic acid activity with fluorescence (Amplex Red Glutamic Acid/Glutamate Oxidase Assay Kit, Molecular Probes). The kit has a range of 0 to 20 μM and a sensitivity of 40 nM. Plasma levels of glutamate were analyzed to determine if the muscle levels obtained from the dialysates were locally produced or possibly emanated from the blood plasma.

To permit estimation of the interstitial tissue concentration of glutamate, the in-vivo relative recovery (RR) of glutamate by the MAB 3 microdialysis probe was measured in a preliminary experiment by adding glutamate (100 μM) to the perfusate in three healthy subjects (one male and two females, 25 to 51 years old) without any symptoms of TMD. It was assumed that the diffusion across the dialysis membrane of glutamate in the perfusate equals that of the diffusion of glutamate from the interstitial fluid. Thus, the RR of glutamate was calculated as $Glu_P - Glu_D/Glu_P$, where Glu_P is the glutamate concentration in the perfusate and Glu_D is the concentration in the dialysate. The mean RR from the three subjects in the preliminary experiment was then used to estimate the interstitial concentration of glutamate (Glu_I) in the masseter muscle of the patients and controls from the glutamate concentration in the dialysate samples (Glu_D) according to the following formula:

$$Glu_I = Glu_D/RR$$

Assessment of Pain and Unpleasantness

A 0 to 10 numeric rating scale (NRS) was used for assessment of current pain intensity during the experiment. This was done 5 minutes prior to insertion of the catheter (baseline) and directly after, as well as 40 minutes and 80 minutes after insertion, with the last measurement made after withdrawing the catheter from the masseter muscle. In addition, the same NRS was used to assess the pain unpleasantness before and after the insertion of the catheter (Fig 1).

McGill Pain Questionnaire (MPQ) and Pain Drawings

The volunteers were asked to fill out a Danish version of the MPQ form,[24,25] and the Pain Rating Index (PRI) (questions 1 through 20) was obtained for statistical analyses. The subjects were also asked to draw the distribution of perceived pain on a lateral view of the face 5 minutes before the insertion of the catheter and after withdrawing the microdialysis probe from the masseter muscle (Fig 1). The pain

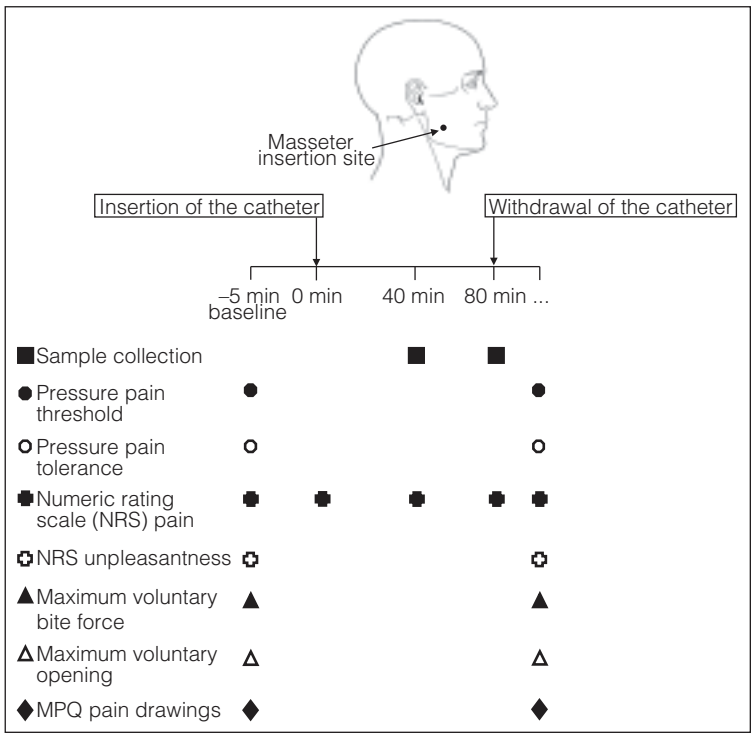

Fig 1 Schematic illustration of the experimental protocol.

area was digitized (Sigma Scan Pro 4.01.003) and expressed as arbitrary units.[12]

Pressure Pain Thresholds (PPTs) and Pressure Pain Tolerances (PPTOLs)

A pressure algometer (Somedic) was used to measure PPTs and PPTOLs (kPa) in response to stimuli applied to the masseter muscles[26] by a single trained experimenter. The subjects kept their jaw at rest and were asked to refrain from clenching their teeth. The pressure was applied to the muscle at a rate of 30 kPa/second with a 1 cm^2 diameter probe, and the volunteers pushed a button when they reached their PPT or PPTOL. The PPT was calculated from the average of three measurements repeated at 1-minute intervals. PPTOLs were assessed from a single measurement at each time point. PPTs and PPTOLs were determined for left and right masseter. One masseter was chosen for the experiment and the other masseter served as an internal control. In patients, the most painful side was chosen for the experiment, while in healthy controls, the side used for the experiment was chosen at random. On the experimental side, the PPTs and PPTOLs were assessed at the site of microdialysis probe insertion before inserting the catheter/microdialysis probe. After withdrawing the microdialysis probe, PPTs and PPTOLs were assessed at a similar anatomical location on masseter muscle that served as the internal control (Fig 1).

Recording of Maximum Voluntary Bite Force (MVB) and Maximum Voluntary Jaw Opening (MVO)

A U-shaped bite force transducer (7 mm high, 1.1 × 1.1 cm area) (Aalborg University) was used to record the MVB between the incisors. During this task, the subjects were encouraged to make their best effort to reach their MVB and, when this was reached, they released the pressure. The MVB (kPa) was determined as the peak value stored on a display.

The MVO was measured with a metallic ruler (mm). Verbal encouragement was given, and the distance between upper and lower incisor was recorded during the maximum effort.

The pain intensity evoked bilaterally by one trial of each of these two tasks was assessed on a 0 to 10 NRS before and after the insertion of the catheter (Fig 1).

Statistical Analyses

Student *t*-tests or Mann-Whitney rank sum tests where data were not normally distributed were used to analyze the primary measurements, ie, levels of glutamate in the dialysates and plasma between groups as well as for the secondary measurements: NRS pain, NRS unpleasantness, MPQ values, pain drawing area, PPTs, PPTOLs, MVB, and MVO. Friedman two-ways repeated measures analysis of

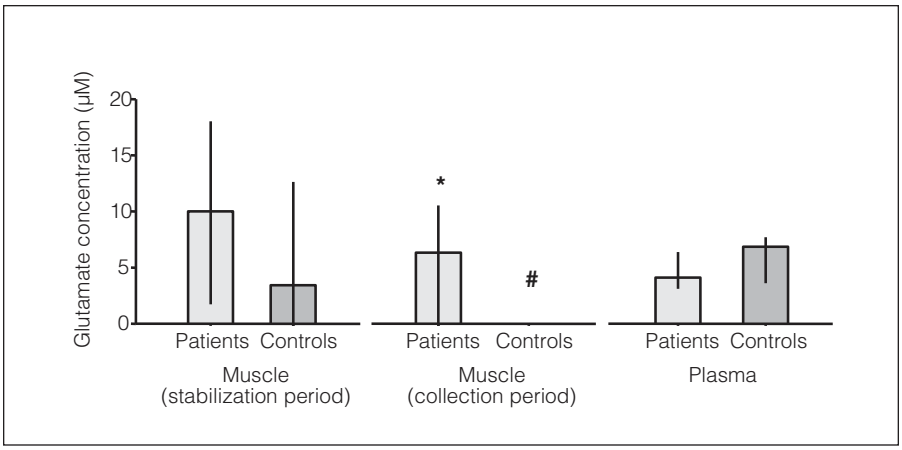

Fig 2 The bar charts illustrate the median concentrations of glutamate in the masseter muscle and in the plasma of myofascial TMD pain patients and healthy controls. There was a significant difference in estimated interstitial concentration of glutamate between the patients and healthy controls during the collection period (*P = .023, Mann-Whitney test). There was a tendency for interstitial glutamate concentrations to decrese in the masseter muscle in the collection period when compared to the stabilization period. In patients, interstitial glutamate concentrations remained elevated above plasma concentrations; however, in healthy controls, glutamate concentration in the muscle was significantly lower than in plasma during the collection period of dialysis (#, P = .025, Friedman test, Dunns post-hoc test). Lines indicate interquartile range.

variance (ANOVA) on ranks, and Dunn's post-hoc analysis when appropriate, were used to compare interstitial and plasma glutamate concentrations. Pearson or Spearman correlation tests were used to determine if there were significant relationships between the intramuscular and plasma glutamate level on one hand and NRS pain intensity, PPT, PPTOL, MPQ, MVB, MVO, NRS unpleasantness, pain drawing areas, or number of muscle sites with pain on palpation (RDC/TMD) on the other hand. The level of significance was set at $P < .05$.

Results

Glutamate Concentrations

The mean (± SD) RR in the preliminary experiment on three healthy subjects was 19% (4.2%). The value of 0.19 was used to estimate the interstitial concentration of glutamate in the masseter muscle of myofascial TMD pain patients and healthy controls from the dialysate glutamate concentration.

There were no significant differences between the patients and healthy control groups in the plasma or interstitial glutamate concentration during the initial 40-minute stabilization period ($P > .153$, t test), but the median concentration of glutamate in the masseter muscle of the myofascial TMD pain patients (7.5 ± 2.6 μM) was significantly higher ($P < .023$, Mann-Whitney test) than the concentration in healthy controls (0.5 ± 0.4 μM) (Fig 2). In the patient group, there was a nonsignificant trend towards higher intramuscular glutamate concentrations during the collection period compared with plasma glutamate concentrations ($P = .052$, Friedman test). In marked contrast, in the healthy controls, the intramuscular concentration of glutamate during the collection period was significantly less than the plasma glutamate concentration ($P = .025$, Friedman test; $P < .05$, Dunn's post-hoc analysis).

The median values for muscle and plasma glutamate concentration for men and women are shown in Table 2. The low number of male patients and controls precluded a statistical analysis for sex-related differences in glutamate concentration, although there was a trend toward higher interstitial glutamate concentration in male patients than in female patients. There was also a general trend towards higher plasma concentrations of glutamate in men than in women.

NRS of Pain and Unpleasantness

There was a statistically significant difference between patients and controls in their reported NRS pain intensity and unpleasantness ($P < .001$, t test). As expected, the patients reported significantly higher levels of pain intensity and unpleasantness (Table 3).

Table 2 Glutamate Concentrations in the Masseter Muscle and Plasma, Median Values (Interquartile Range)

	TMD pain patients		Healthy controls	
	Women (n = 10)	Men (n = 3)	Women (n = 8)	Men (n = 2)
Stabilization period	8.6 μM (2.1–14.2)	31.1 μM (7.8–32.2)	6.8 μM (0.0–13.4)	1.8 μM (0.0–3.9)
Collection period	3.9 μM (0.0–8.9)	13.7 μM (3.4–28.7)	0.0 μM (0.0–1.1)	0.0 μM (0.0–0.0)
Plasma glutamate	3.8 μM (3.1–5.5)	6.9 μM (4.3–10.7)	6.0 μM (3.4–7.5)	10.3 μM (7.7–13.0)

Table 3 Pain Characteristics, Mean Values (SEM)

	TMD pain patients (n = 13)	Healthy controls (n = 10)	*P* value	Test
NRS pain intensity (0 to 10)				
5 min prior to insertion of the catheter/probe	3.1 (0.4)	0.0 (0.0)	< .001*	Mann-Whitney
Immediately after insertion of the catheter/probe	3.5 (0.4)	1.7 (0.6)	.028*	Mann-Whitney
40 min after insertion of the catheter/probe	2.9 (0.5)	0.3 (0.2)	< .001*	Mann-Whitney
80 min after insertion of the catheter/probe	3.0 (0.5)	0.1 (0.1)	< .001*	Mann-Whitney
Immediately after withdrawal of the probe	2.9 (0.5)	0.1 (0.1)	< .001*	Mann-Whitney
NRS pain unpleasantness (0 to 10)				
5 min prior to insertion of the catheter/probe	3.8 (0.6)	0.0 (0.0)	< .001*	Mann-Whitney
Immediately after removal of the catheter/probe	3.1 (0.5)	0.2 (0.1)	< .001*	Mann-Whitney
Pain drawing area (arbitrary units)				
5 min prior to insertion of the catheter/probe	125.6 (24.4)	0.0 (0.0)	< .001*	Mann-Whitney
Immediately after removal of the catheter/probe	68.0 (14.4)	1.7 (1.7)	.001*	Mann-Whitney
PRI MPQ total scores (0 to 112)				
5 min prior to insertion of the catheter/probe	21.5 (4.0)	0.0 (0.0)	< .001*	Mann-Whitney
Immediately after removal of the catheter/probe	16.9 (3.0)	0.0 (0.0)	< .001*	Mann-Whitney

Table 4 Mechanical Sensitivity Characteristics, Mean Values (SEM)

	TMD pain patients (n = 13)	Healthy controls (n = 10)	*P* value	Test
PPT (kPa)				
5 min prior to insertion of the catheter/probe	131.7 (11.7)	172.0 (30.9)	.264	Mann-Whitney
Immediately after removal of the catheter/probe	118.6 (19.4)	131.1 (25.1)	.692	*t* test
PPTOL (kPa)				
5 min prior to insertion of the catheter/probe	277.4 (31.4)	404.9 (54.7)	.045*	*t* test
Immediately after removal of the catheter/probe	228.0 (30.9)	308.9 (59.5)	.239	Mann-Whitney

MPQ and Pain Drawings

The patients had significantly higher PRI MPQ scores and reported a larger pain area on their pain drawings than the controls (Mann-Whitney test, $P < .001$) (Table 3).

PPTs and PPTOLs

There were no significant differences on either side in PPTs between patients and controls ($P > .263$). The PPTOLs were significantly lower in the patients than in the controls before ($P = .045$, *t* test) but not immediately after the removal of the catheter/microdialysis probe ($P = .239$, Mann-Whitney test) (Table 4).

Table 5 Functional Characteristics, Mean Values (SEM)				
	TMD pain patients (n = 13)	Healthy controls (n = 10)	*P* value	Test
MVB (kPa)				
5 min prior to insertion of the catheter/probe	18.8 (1.8)	22.7 (2.6)	.229	*t* test
Immediately after removal of the catheter/probe	18.0 (1.9)	23.0 (2.7)	.145	*t* test
MVO (mm)				
5 min prior to insertion of the catheter/probe	48.0 (2.6)	52.1 (1.9)	.226	Mann-Whitney
Immediately after removal of the catheter/probe	47.8 (2.6)	51.6 (2.1)	.368	Mann-Whitney

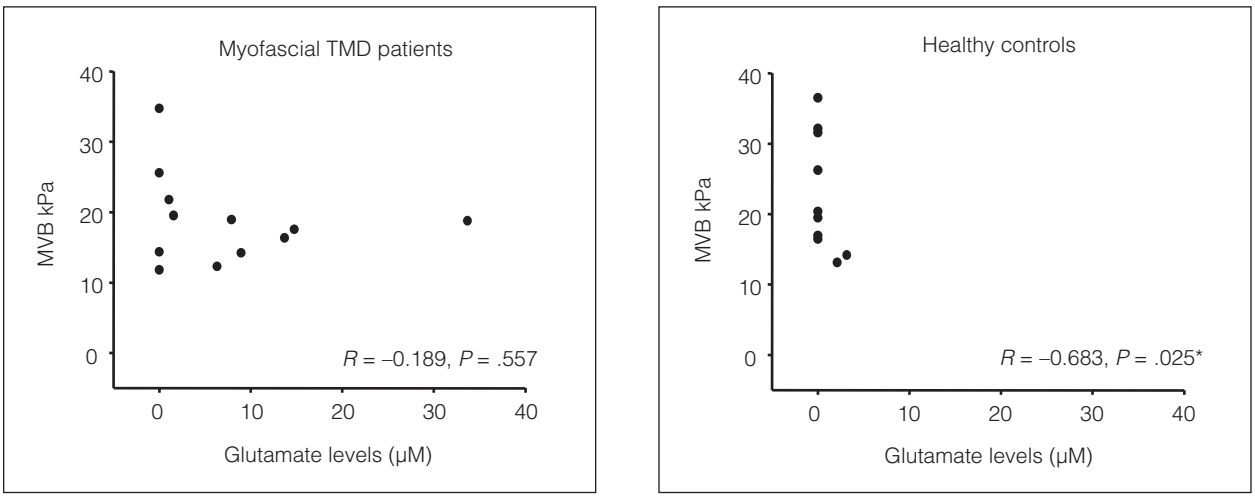

Fig 3 The scatter plot shows the negative correlation between the maximum voluntary bite force (MVB) and the estimated interstitial concentration of glutamate in the masseter muscle for myofascial TMD pain patients (n = 13) and healthy volunteers (n = 10). Correlations reached significance in the healthy control group (*).

MVB and MVO

There were no significant differences between patients and controls in MVB ($P > .144$, t test) or MVO ($P > .225$, Mann-Whitney test) (Table 5).

Correlations

In the myofascial TMD pain patients, there was a significant correlation between the glutamate levels in the stabilization and collection periods ($R = 0.884$, $P < .001$ Pearson test), but there were no significant correlations between interstitial and plasma glutamate levels. No significant correlations between dialysate or plasma glutamate levels and baseline NRS pain intensity values were identified for patients. No significant correlation between interstitial glutamate during the stabilization period and the NRS pain intensity reported immediately after the insertion of the catheter/microdialysis probe was found for either group. There was, however, a significant inverse correlation between interstitial glutamate during the collection period and MVB in the healthy control group ($R = –0.683$, $P = .025$, Spearman test) (Fig 3). In the patient group, interstitial glutamate concentration was inversely correlated with pain drawing areas ($R = –0.569$, $P = .042$, Pearson test) (Fig 4a). Plasma glutamate concentration was inversely correlated with the number of muscle sites with pain on palpation (RDC/TMD) ($R = –0.636$, $P = .019$, Pearson test) (Fig 4b). There were no other significant correlations.

Discussion

This is the first report of interstitial glutamate concentrations in the masseter muscle in healthy humans and/or in patients with chronic myofascial TMD pain. The results demonstrate that there is a significantly elevated concentration of interstitial glutamate in the masseter muscle of myofascial

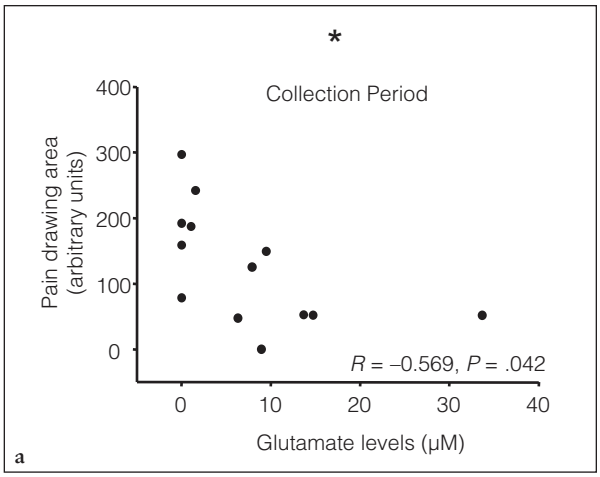

Fig 4a The scatter plot shows the significant (*) negative correlation between the pain drawing area and the estimated interstitial concentration of glutamate in the masseter muscle for myofascial TMD pain patients (n = 13).

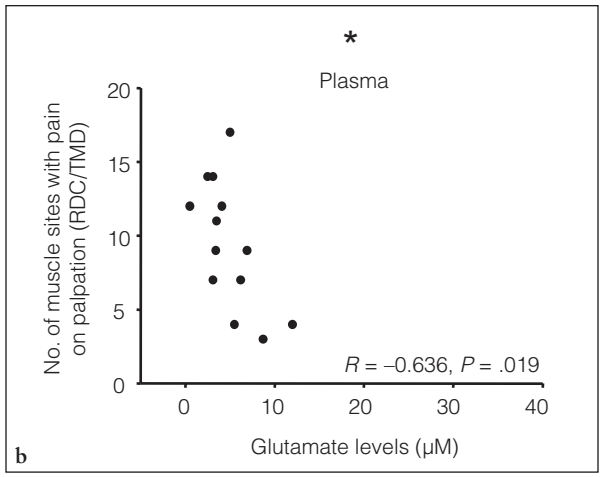

Fig 4b The scatter plot shows the significant (*) negative correlation between the number of muscle sites with pain on palpation (RDC/TMD) and plasma glutamate concentration for patients (n = 13).

TMD pain patients compared to healthy individuals. This finding identifies a specific change in the interstitial environment of the masseter muscle associated with chronic myofascial TMD pain and supports the view that local release of algogenic substances in the masseter muscle may be involved in the pathophysiology of persistent myofascial TMD pain in this condition. However, there were no significant correlations between pain rating scores and interstitial or plasma glutamate concentrations in the myofascial TMD pain patients; this finding is consistent with a previous study that reported a decrease in pain intensity but not in glutamate concentration after treatment of Achilles tendons.[27] It is possible that elevated concentrations of interstitial glutamate found in the masseter muscle of myofascial TMD pain patients reflect a more global metabolic change in the masseter muscle tissue and thus are not directly responsible for masseter muscle pain. However, given the invasive nature of the microdialysis procedure, it was understandably challenging to recruit patients into this study, and thus the inability to significantly correlate pain and interstitial glutamate concentrations could be due to the fairly low number of patients enrolled.

Interstitial Glutamate Concentration and Deep Tissue Pain

The findings of elevated glutamate levels in the masseter muscle of myofascial TMD pain patients in the present study are consistent with previous studies that have shown elevated tissue concentrations of glutamate in chronic, noninflammatory deep tissue pain conditions of muscles and tendons elsewhere in the body.[6,28,29] Glutamate concentrations of ~200 μM have been reported in the extensor carpi radialis brevis tendon of patients with tennis elbow and the patellar tendon of patients with "jumpers' knee," as compared to 50 to 70 μM for healthy controls.[28–30] Similarly, trapezius muscle glutamate concentrations in chronic work-related myalgia were reported to be 47 μM versus 36 μM for healthy controls.[6] The difference between patients and controls in the present study is similar to what was found in tendon tissue.[28–30] Although our estimates of interstitial glutamate concentrations in the masseter muscle of myofascial TMD pain patients and healthy controls are lower than those reported for tendons and the trapezius muscle, this disparity is likely due to methodological differences such as the type of microdialysis probe employed (ie, membrane length and diameter), the perfusion rate, or the method employed to measure glutamate concentration in the dialysate. Such methodological differences can result in significant differences in the estimated concentrations of glutamate. For example, the interstitial concentration of glutamate in the rat masseter muscle measured directly with a glutamate biosensor was 24 μM, whereas when measured using microdialysis, it was estimated to be ~65 μM.[31] Nevertheless, what is consistent in most human studies is that interstitial glutamate concentrations in deep tissue associated with clinical noninflammatory pain are one and a

half to four times greater than in pain-free controls. It should be mentioned that a previous study[32] could not detect differences in glutamate levels in trapezius tender points of patients with chronic tension-type headache compared to healthy controls but, in contrast to the present study where all myofascial TMD pain patients had ongoing pain in the masseter muscle, there was no spontaneous pain in the trapezius muscle in the tension-type headache patients.

It could be argued that the present finding of increased glutamate interstitial levels in the masseter muscle of TMD patients compared with healthy controls was partly due to differences in placement of the probe. Previous research suggest that the concentrations of certain inflammatory mediators are elevated in tender areas of muscle when compared with nontender muscle.[33] Indeed, the authors chose to examine the most painful sites of the masseter muscle with the expectation that they would be associated with elevated concentrations of glutamate. It is, however, unknown whether interstitial glutamate concentrations in the healthy masseter muscle are uniform throughout the muscle or vary with anatomical location. Moreover, magnetic resonance imaging or ultrasound[6] would be one way to verify and standardize the location of the probe inside the masseter muscle, but the inclusion of this technique in the current study was not possible. What is apparent in the current study is that there were very large interindividual differences in interstitial glutamate concentration, even in healthy subjects where the probe was put into a standard position in the masseter muscle. These interindividual differences appear far greater than the differences previously reported for other inflammatory mediators at tender and nontender sites within the same muscle.[33] Yet, even with these large interindividual differences, there was a significant difference in estimated interstitial glutamate concentrations between patients and healthy controls.

The mechanism(s) responsible for elevated interstitial concentrations of glutamate in tender areas of the masseter muscles of TMD pain patients cannot be determined from the present study. It may be that greater trauma, which was introduced as a result of insertion of the probe into already painful areas in the patients, resulted in a greater release of glutamate. However, there was no significant difference in the estimated interstitial glutamate concentration in patients and controls during the first dialysis period after insertion of the probe. While it is possible that glutamate clearance is different in patients and controls, it is equally possible that an increased release of glutamate into the interstitial space of the patients might underlie the observed difference in interstitial glutamate concentrations during the second dialysis period. Moreover, there might be differences in the reuptake of glutamate by muscle cells.[34] Further investigations are required to determine the mechanism(s) that might contribute to elevated interstitial glutamate concentrations of glutamate in the masseter muscles of TMD pain patients.

It has been determined that in the rat masseter muscle, a two to three times elevation of interstitial glutamate concentrations over baseline is associated with excitation and mechanical sensitization of slowly conducting masseter afferent fibers.[31] This suggests that elevated concentrations of glutamate in the masseter muscle of myofascial TMD pain patients could be part of the pathophysiological mechanisms related to ongoing pain and mechanical sensitivity, which are characteristic features of this condition.

Role of Peripheral Glutamate Receptors

There is ample evidence that injection of glutamate into the masseter muscle of human subjects evokes muscle pain, induces a period of mechanical sensitization, and alters jaw-stretch reflex responses.[8,10,11,14,35] Anatomical evidence indicates that the NMDA receptor, a glutamate receptor subtype, is found in neural structures in human tendon tissue samples[30,36] and is expressed by afferent fibers that innervate the rat masseter muscle.[16] Indeed, glutamate-evoked masseter muscle pain and mechanical sensitization can be attenuated by local administration of the NMDA receptor antagonist ketamine.[7,15] However, there is considerable variability in the efficacy of ketamine to attenuate glutamate-evoked pain and mechanical sensitization in the masseter muscle, which may be an indication that other peripheral receptor mechanisms are involved.[10] Animal studies suggest that peripheral non-NMDA receptors could also contribute to glutamate-induced mechanical sensitization of the masseter muscle[37] and that elevated interstitial levels of glutamate in the masseter muscle may result in the release of neuropeptides.[38] These data suggest that elevated glutamate concentrations in the masseter muscle of human subjects could contribute to muscle pain and sensitization by either directly activating peripheral NMDA and/or other peripheral glutamate receptor subtypes or indirectly through the release of other neuroactive agents.

Clinical Relevance

Despite evidence that interstitial glutamate concentrations are elevated in relevant deep tissues in association with ongoing pain and sensitization, it is

uncertain whether treatment directed against glutamate or its receptors will provide clinically relevant analgesia. In a study to determine if eccentric training would decrease pain and intratendinous glutamate concentrations in patients with chronic Achilles tendinosis, it was reported that although patients were pain-free after treatment, the high intratendinous glutamate concentrations were unchanged.[27] Furthermore, a recent randomized clinical trial of local injections of ketamine into the masseter muscle of myofascial TMD pain patients found large interindividual differences in response but no significant overall treatment effect on muscle pain or sensitivity.[39] However, while it remains to be determined whether there is a direct cause and effect relationship between elevated interstitial concentrations of glutamate and noninflammatory deep tissue pain, the present finding of elevated concentrations of interstitial glutamate in the masseter muscle of myofascial TMD pain patients suggests that there may be a significant peripheral component to the ongoing muscle pain and sensitivity that characterize this disorder and that may cause peripheral sensitization resulting in reduced PPTs and PPTOLs.

The finding of a negative correlation between MVB and masseter muscle glutamate concentration in the healthy controls was unexpected. This suggests that even in healthy controls there may be a relationship between masseter muscle glutamate concentration and jaw muscle function; however, additional studies in larger populations will be needed to confirm this observation. Moreover, the finding that MVO and MVB are not significantly different between healthy volunteers and TMD patients may be a result of the small number of patients and healthy controls examined in this study.

On the other hand, the inverse relationship between pain drawing area and glutamate levels in myofascial TMD pain patients might suggest that in individuals with very localized pain, glutamate plays a more important role, whereas other mechanisms are more important in patients with more diffuse (ie, larger pain areas) TMD pain. Moreover, the negative correlation of the number of muscle sites with pain on palpation (RDC/TMD) and the plasma levels of glutamate suggests that higher levels of systemic glutamate in TMD pain patients are not playing an important role in the clinical pain symptoms within the craniofacial area.

Conclusions

The present study demonstrates that elevated concentrations of interstitial glutamate are found in the masseter muscle of myofascial TMD pain patients. These findings are consistent with previous reports of elevated glutamate concentrations in conditions with chronic tendon pain and myalgia[6,28,29] and suggest that peripherally released glutamate could be involved in the pathophysiology of myofascial TMD pain.

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