Pain and Intramuscular Release of Algesic Substances in the Masseter Muscle After Experimental Tooth-Clenching Exercises in Healthy Subjects

Andreas Dawson, DDS, Odont Dr
Postgraduate Student
Department of Orofacial Pain and Jaw Function
Faculty of Odontology
Malmö University, Sweden

Bijar Ghafouri, PhD
Associate Professor
Department of Medicine and Health Sciences
Faculty of Health Sciences
Linköping University and Pain and Rehabilitation Centre
and Department of Clinical and Experimental Medicine
Faculty of Health Sciences
Linköping University and Centre of Occupational and Environmental Medicine
Östergötland County Council
Linköping, Sweden

Björn Gerdle, MD, PhD
Professor
Department of Medicine and Health Sciences
Faculty of Health Sciences
Linköping University and Pain and Rehabilitation Centre
Östergötland County Council
Linköping, Sweden

Thomas List, DDS, Odont Dr
Professor, Department of Orofacial Pain and Jaw Function
Faculty of Odontology
Malmö University
Malmö, Sweden
Department of Rehabilitation Medicine
Skåne University Hospital
Lund, Sweden

Peter Svensson, DDS, Dr Odont, PhD
Professor, Section of Clinical Oral Physiology
Department of Dentistry
Aarhus University
and Center for Functionally Integrative Neuroscience (CFIN)
MindLab
Aarhus University Hospital, Denmark

Malin Ernberg, DDS, PhD
Professor
Section of Orofacial Pain and Jaw Function
Department of Dental Medicine
Karolinska Institutet
Huddinge, Sweden

Correspondence to:
Dr Andreas Dawson
Department of Orofacial Pain and Jaw Function
Faculty of Odontology
Malmö University
SE -205 06 Malmö, Sweden
Email: andreas.dawson@mah.se

Aims: *To investigate whether experimental tooth clenching leads to a release of algesic substances in the masseter muscle.* ***Methods:*** *Thirty healthy subjects (16 females, 14 males) participated. During two sessions, separated by at least 1 week, intramuscular microdialysis was performed to collect masseter muscle 5-hydroxytryptamine (5-HT) and glutamate as well as the metabolic markers pyruvate and lactate. Two hours after the start of microdialysis, participants were randomized to a 20-min repetitive experimental tooth-clenching task (50% of maximal voluntary contraction) or a control session (no clenching). Pain and fatigue were measured throughout. The Friedman and Wilcoxon tests were used for statistical analyses.* ***Results:*** *No alterations were observed in the concentrations of 5-HT, glutamate, pyruvate, and lactate over time in the clenching or control session, or between sessions at various time points. Pain (*$P < .01$*) and fatigue (*$P < .01$*) increased significantly over time in the clenching session and were significantly higher after clenching than in the control session (*$P < .01$*).* ***Conclusion:*** *Low levels of pain and fatigue developed with this experimental tooth-clenching model, but they were not associated with an altered release of 5-HT, glutamate, lactate, or pyruvate. More research is required to elucidate the peripheral release of algesic substances in response to tooth clenching.* J OROFAC PAIN 2013;27:350–360. doi: 10.11607/jop.1170

Key words: bruxism, glutamate, masseter muscle, microdialysis, serotonin, temporomandibular disorders

Chronic pain in general is a major health concern that has negative economic effects on society and affects patients in both their social and their working lives.[1] Myofascial temporomandibular disorder (TMD) is more frequently reported in females than in males, and its prevalence in the general population is approximately 10%.[2] Soreness and pain in the masticatory muscles, limited jaw function, and increased pain upon function are symptoms commonly manifested in this condition.[3,4] Little is known about the underlying mechanisms, but both peripheral and central mechanisms are thought to be implicated in the pathophysiology of myofascial TMD.[5]

Studies have shown that self-reported tooth clenching is a risk factor for myofascial TMD and could lead to an overloading of the masticatory muscles and development of pain.[6] Muscle overload may be associated with disturbed local blood flow, impaired microcirculation, and development of relative ischemia,[7] thus possibly facilitating the release of algesic substances that sensitize and excite muscle nociceptors.[8]

Noxious mechanical stimuli and algesic substances, such as the neurotransmitters 5-hydroxytryptamine (5-HT) and glutamate, can activate and sensitize muscle nociceptors.[8–10] It has also been shown that in response to tissue damage or in ischemic conditions, peripherally released 5-HT can provoke the release of glutamate and substance P,[8,11] thus amplifying pain. There is also evidence from animal models of craniofacial pain that glutamate is involved in pain-related hypersensitivity and trigeminal central sensitization,[12–14] and both glutamate and 5-HT induce pain and hyperalgesia when injected into a healthy muscle.[15,16]

It has been suggested that the predominance of TMD in females might be ascribed to their higher stress levels.[17] Female TMD patients have a significantly higher level of cortisol during daytime than healthy controls.[18] Estrogen and cortisol are known to interact with the central release of 5-HT and glutamate,[19–21] while their influence on the peripheral release of 5-HT and glutamate is unknown. If so, it is possible that females have a higher peripheral release of 5-HT than males due to higher levels of cortisol and estrogen.

Patients with masseter and trapezius myalgia are reported to exhibit significantly higher levels of muscle 5-HT and glutamate compared to healthy subjects.[22–25] It was also observed that there was a shift towards an anaerobic metabolism in patients with trapezius myalgia, as indicated by higher muscle levels of pyruvate and lactate.[25] These metabolic substances have been suggested to be an indicator of reduced tissue oxygenation.[26–28] In response to low-force exercise, patients with trapezius myalgia and healthy subjects exhibit increased levels of glutamate, lactate and pyruvate,[25] but significant time effects for 5-HT have not been observed.[24]

So far, no previous study has investigated the intramuscular events that might occur in the human masseter muscle in response to experimental tooth clenching. Therefore, this study aimed to investigate whether experimental tooth clenching leads to a release of algesic substances in the masseter muscle. The following hypotheses were tested: *(1)* experimental tooth clenching increases masseter muscle interstitial levels of 5-HT, glutamate, pyruvate, and lactate as well as blood flow; *(2)* in response to experimental tooth clenching, females have a significantly higher release of 5-HT, glutamate, pyruvate, and lactate compared to males; and *(3)* the peripheral release of 5-HT and glutamate is correlated with salivary cortisol and β-estradiol.

Materials and Methods

Subjects

Thirty pain-free subjects participated in the study, 16 females (mean age ± SD: 36 ± 16 years) and 14 males (mean age ± SD: 41 ± 18 years). All subjects were recruited from Malmö University and screened per the Research Diagnostic Criteria for TMD.[4]

Exclusion criteria were: age ≤ 18 years, systemic inflammatory connective tissue diseases (eg, rheumatoid arthritis), whiplash-associated disorders, chronic muscle pain conditions (eg, fibromyalgia), neuropathic pain or neurological disorders (eg, oromandibular dystonia), pain of dental origin, pregnancy or lactation, high blood pressure, anticoagulant treatment, ongoing dental treatment, extensive restorations (eg, full bridges, dentures), allergy to antibiotics, allergy to prilocaine or lidocaine, current use of analgesics (eg, paracetamol, nonsteroidal anti-inflammatory drugs, salicylate drugs, and opioids, or other medication that would influence pain perception, [eg, antidepressants or antiepileptic drugs]), and severe skeletal malocclusions. Exclusion criteria were assessed in each subject by collecting their medical history.

This study was conducted in the Department of Orofacial Pain and Jaw Function at Malmö University, Malmö, Sweden. The Regional Ethics Review Board at Lund University approved the project and subject selection (2010/31). The Declaration of Helsinki guidelines were followed. Before entering this study, subjects signed an informed consent form and were informed that they could withdraw from the experiment at any time, with no consequences. Subjects were financially compensated upon completion of their participation.

Study Design

This study was designed as a single-blind randomized crossover trial comprising two sessions, each session lasting 4 hours. Figure 1 is a schematic illustration of the study design. To avoid carryover effects, intervals between sessions were a minimum of 1 week. In both sessions, intramuscular microdialysis was performed on the right masseter muscle to sample 5-HT, glutamate, lactate, and pyruvate. Subjects were randomly allocated (random number generator, SPSS) to a repetitive experimental tooth-clenching exercise or a control session with no clenching.

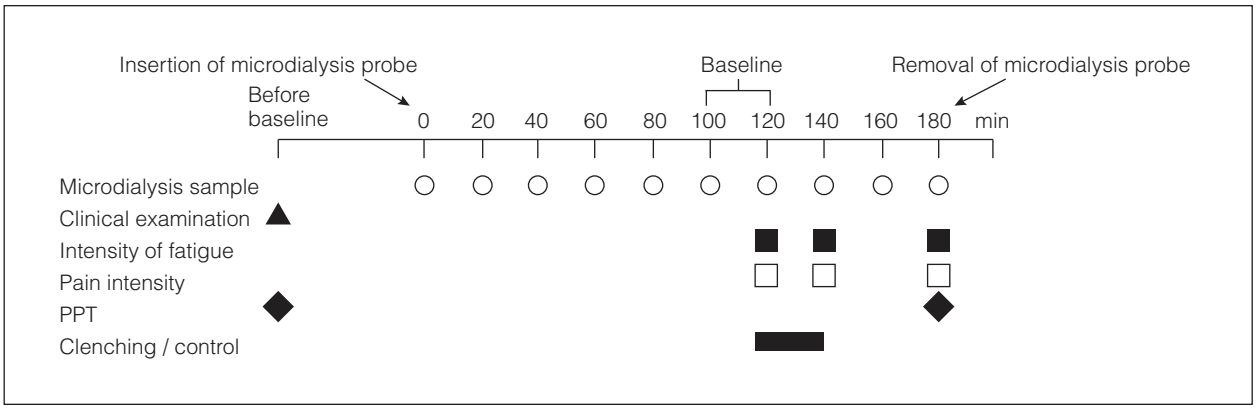

Fig 1 Schematic illustration of the study design. Intramuscular microdialysis was conducted to sample 5-HT, glutamate, pyruvate, and lactate. Two hours after the start of microdialysis, the subjects were randomized to a repetitive tooth-clenching task, or a control task. Pain intensity and fatigue were measured at baseline, after the clenching and the control task, and after recovery. Pressure pain threshold (PPT) was assessed before insertion of the microdialysis probe and after the removal of the microdialysis probe.

On the experimental day in each session, subjects brought a saliva sample, which they had collected immediately after awakening, to the clinic for further analysis of salivary cortisol. Directly before insertion of the microdialysis catheter (in each session), a venous blood sample was collected for further analysis of β-estradiol. Before insertion of the microdialysis probe (in each session, before baseline), maximal voluntary clenching force (MVCF) and pressure pain threshold (PPT) were assessed. A 120-minute stabilization period directly followed probe insertion, the last 20 minutes of which served as a baseline measure of the microdialysates. After stabilization, baseline assessments of pain intensity and fatigue were made, followed by a 20-minute clenching or control (relaxation of the masticatory muscles) session and 40 minutes of rest (recovery). Assessments of pain intensity and fatigue were repeated directly after the clenching or control session and after recovery. PPT was measured after removal of the microdialysis probe.

Subjects sat upright in a dental chair with head support throughout the experiment and were instructed to relax their masticatory muscles—except during the experimental tooth-clenching exercises. One operator made all measurements.

Experimental Tooth Clenching and Control Condition

A bite-force transducer (Aalborg University, Denmark), placed between the molars on the right side, was used to assess MVCF (kg). Three times, the subjects were asked to bite down on the transducer (clench) as intensely as possible for 2 to 3 seconds. Mean MVCF was calculated from the three registrations. The 20-minute experimental tooth-clenching session then began. Subjects did 20 bouts of clenching at 50% of their mean MVCF. Each bout lasted 30 seconds with 30-second intervals between bouts.[29] The bite-force transducer displayed the clenching force, and subjects were instructed to maintain 50% mean MVCF by continuously watching the display.

During the control session, subjects were instructed to relax their masticatory muscles for 20 minutes.

Saliva Sampling

After fasting overnight, subjects used a Salimetrics Oral Swab (SOS; Salimetrics) to collect unstimulated saliva on the morning of the experimental day. Subjects were instructed to place the SOS under the tongue for 2 minutes immediately after waking up and then to place the swab in a Salimetrics Swab storage tube. When the subjects arrived at the clinic, the samples were immediately frozen (–70°C). On the day of cortisol analysis, the saliva samples were brought to room temperature and centrifuged (1,500 g, +20°C) for 15 minutes to precipitate mucins. The commercially available Salimetrics High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit was used according to the manufacturers' instructions to analyze salivary cortisol.

Blood Sampling

Venous blood samples were collected with a Vacutainer (BD Vacutainer Safety-Lock; Becton, Dickinson, and Company) in a 3-mL plasma separation

tube for analysis of β-estradiol. Immediately after collection, the samples were centrifuged (2,000 g, +20°C) for 10 minutes, and the plasma was pipetted to 1-mL Eppendorf tubes and stored at –70°C. The Laboratory of Clinical Chemistry at the Skåne University Hospital in Malmö analyzed β-estradiol with the commercially available Delfia Estradiol Kit (Wallac OY). The use of oral contraceptives and the phase of the menstrual cycle were not taken into consideration during blood sampling.

Assessment of Pressure Pain Threshold

An electronic algometer (Somedic Sales AB) was used to assess PPT, defined as the amount of pressure (kPa) needed to produce a sensation of pain. A 1-cm^2 probe was applied to the mandibular attachment of the right masseter muscle with a constant pressure of 30 kPa/s. The mean of three measurements, made at 60-second intervals, was calculated. Acceptable reliability was previously found for PPT measured on the masseter muscle.[30]

Assessment of Pain and Fatigue

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

Microdialysis

Intramuscular microdialysis was conducted 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) for 20 minutes. A standard catheter (diameter 1.3 × 32 mm, BD Venflon Pro; Becton Dickinson Infusion Therapy AB) was inserted into the center of the masseter belly at a 45-degree angle to the skin and a length 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 (diameter 0.5 mm; membrane length 10 mm; shaft length 20 mm; molecular cut-off 6 kDa, MAB11.20.10; Microbiotech/se) 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.[31] 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 a Ringer-acetate (Baxter Viaflo, Baxter Medical AB) solution containing 0.5 mM Ringer-lactate (Baxter Viaflo, Baxter Medical AB), and 3 mM glucose (Glucose 50 mg/mL; B. Braun Melsungen AG) to prevent the interstitial space from draining.[32] Three μM [^{14}C]-lactate (specific activity: 7.4 MBq/mL; PerkinElmer Life Sciences) was added to the Ringer-acetate solution to determine relative in vivo recovery (RR) and allow determination of the interstitial concentrations of pyruvate, glutamate, lactate, and 5-HT, and of 3 μM ^3H_2O (specific activity: 37 MBq/mL; PerkinElmer Life Sciences) to estimate nutritive blood flow.[25,33,34] The outlet tubing of the microdialysis probe was placed in a 500-μL microvial.

Microdialysates were sampled every 20 minutes during 3 hours and were stored at –70°C. Only the samples collected at baseline (100 to 120 minutes), during the clenching or control task (120 to 140 minutes), and during the last 20 minutes of the recovery period (160 to 180 minutes) were later analyzed for algesic substances.

Analyses

Concentrations of glutamate, lactate, and pyruvate were analyzed with an ISCUS Clinical Microdialysis Analyzer (Dipylon Medical AB). 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 the 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; Ghafouri et al[24] described this methodology previously. The LOD for 5-HT was 20 fmol/10 μL. The PAINOMICS lab at Linköping University Hospital analyzed all microdialysates.

Dialysate or perfusate (5 μL) was pipetted into a counting vial containing 3 mL scintillation fluid (High-flash Point, Universal LSC-Cocktail, ULTIMA GOLD, PerkinElmer) and vortexed; β-counting was done in a liquid scintillation counter (Beckman LS 6000TA; Beckman Instruments). The RR for lactate was calculated for each sample: $(cpm_p - cpm_d)/cpm_p$, where cpm_p was counts per minute of perfusate and cpm_d was counts per minute of dialysate. The interstitial levels (Ci) of the algesic substances were 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.

Table 1 Mean ± SD Maximal Voluntary Clenching Force (MVCF; kg), Salivary Cortisol (μg/dL), and Plasma β-Estradiol (pmol/L)

	Baseline	
	Clenching	Control
MVCF		
All	52.7 ± 18.6	55.8 ± 18.8
Females	50.2 ± 16	54.1 ± 16.7
Males	55.5 ± 21.1	57.8 ± 21.4
Salivary cortisol		
All	0.58 ± 0.44	0.54 ± 0.32
Females	0.60 ± 0.37	0.70 ± 0.32[aa]
Males	0.58 ± 0.54	0.36 ± 0.23
Plasma β-estradiol		
All	181 ± 236	186 ± 271
Females	262 ± 303[a]	274 ± 351[a]
Males	88 ± 27	85 ± 33

Significant sex difference within the same session ([a]$P < .05$ and [aa]$P < .01$).

Nutritive blood flow was estimated: $1/(cpm_d/cpm_p)$ for ^3H_2O, where cpm_d was ^3H_2O counts per minute in the dialysate, and cpm_p in the perfusate.

Statistical Analyses

The Statistical Package for the Social Science for Windows, version 17 (SPSS, IBM) was used for all calculations. Means and SDs were calculated for age and MVCFs. An independent samples *t* test compared age differences between sexes. The Kolmogorov-Smirnov test assessed data for normality. MVCF, salivary cortisol, β-estradiol, and RR were normally distributed, so parametric statistics could be used. The paired samples *t* test assessed mean values of salivary cortisol and β-estradiol for significant differences between sessions, and the independent samples *t* test assessed between-sex differences in each session.

Repeated measures analysis of variance assessed RR for differences between sessions and over time. A paired samples *t* test assessed differences in mean MVCF between sessions, and an independent samples *t* test assessed sex differences. Pain intensity; fatigue; interstitial levels of 5-HT, glutamate, lactate, and pyruvate; and PPT were not normally distributed; after transformation, the data were still not normally distributed, so nonparametric statistics were used. The Friedman test analyzed within-session differences in interstitial levels of mediators and for pain intensity and fatigue. The Wilcoxon signed rank test was applied in the post-hoc analysis. To avoid risk of type I errors, the Bonferroni correction was applied.

Between-session comparisons at various time points were made with the Wilcoxon signed rank test and a Bonferroni correction was applied. The Mann-Whitney *U* test was used to analyze differences between sexes at various time points, and to evaluate the significance of changes in 5-HT and glutamate between self-reported bruxers and participants without awareness of bruxism at various time points in each session. Spearman correlation test with Bonferroni correction analyzed significant correlations between pain intensity after experimental tooth clenching, PPT, 5-HT, glutamate, pyruvate, lactate, and cortisol or β-estradiol. A power calculation showed that 12 subjects of each sex were sufficient to detect a difference in mean dialysate levels of 0.8 SD when $\alpha = .05$ and $\beta = .80$. All statistical analyses were performed two-tailed at a significance level of 5%.

Results

Baseline Values of Maximal Voluntary Clenching Force, Cortisol, and β-Estradiol

Table 1 illustrates the results of MVCF, salivary cortisol, and β-estradiol in a comparison between sessions, and between sexes in each session. Significant differences for MVCF were not observed between sessions, nor between sexes within each session. Females had a significantly higher level of salivary cortisol than males in the control session ($P < .01$). The level of β-estradiol was significantly higher for females than males in both sessions ($P < .05$).

Pressure Pain Threshold

In the clenching session, PPT was significantly lower after microdialysis compared to baseline ($P < .01$), whereas PPT was unchanged in the control session. No significant between-session differences occurred at any time point (Table 2).

In females, PPT was significantly decreased following the clenching session ($P < .01$), but unaltered following the control session. In males, no significant changes in PPT were observed following any session. Significant between-session differences were not observed at any time point for either sex.

Males had significantly higher PPT than females at baseline and after microdialysis ($P < .01$) in the clenching session, and a significantly higher PPT at baseline ($P < .05$) in the control session.

Table 2 Median (Interquartile Range) PPT (kPa), Pain Intensity, and Intensity of Fatigue (Both: 0–100-mm VAS) Measured at Baseline, After the Clenching and Control Sessions, and After Recovery

	Baseline		After clenching/control task		After recovery	
	Clenching	Control	Clenching	Control	Clenching	Control
PPT						
All	164 (77)	160 (66)	–	–	141 (59)[a]	172 (73)
Females	149 (48)	146 (74)	–	–	121 (39)[b]	159 (62)
Males	207 (111)[cc]	194 (71)[c]	–	–	168 (75)[cc]	191 (120)
Pain intensity						
All	0 (10)	0 (10)	15 (30)[dd]	0 (10)	10 (20)	5 (12.5)
Females	0 (10)	0 (10)	25 (27.5)[d]	0 (7.5)	0 (20)	10 (20)
Males	0 (2.5)	0 (10)	10 (32.5)	0 (10)	10 (15)	0 (10)
Intensity of fatigue						
All	0 (10)	0 (2.5)	40 (32.5)[dd]	0 (2.5)	10 (20)[d]	0 (10)
Females	0 (10)	0 (0)	40 (17.5)[dd]	0 (0)	10 (17.5)[d]	0 (10)
Males	0 (2.5)	0 (10)	45 (42.5)[dd]	0 (12.5)	10 (35)	0 (2.5)

[a]Significant decrease compared with baseline in the same session ($P < .01$). [b]Significant decrease over time in the same session ($P < .01$). Significant sex difference within the same session and time point ([c]$P < .05$ and [cc]$P < .01$). Significant difference compared to the control session at the same time point ([d]$P < .01$, and [dd]$P < .001$).

Pain Intensity and Fatigue

Table 2 shows the pain intensity and fatigue at the various time points for each session and sex. Pain intensity increased significantly over time in the clenching session ($P < .001$), with significantly higher levels of pain after the clenching task compared to baseline ($P < .01$). In the control session, pain intensity changed significantly over time ($P < .05$), but at which time points could not be detected. In a between-session comparison, pain intensity was significantly higher after the clenching task than after the control task ($P < .01$).

No significant between-sex differences in pain intensity occurred at any time point in either session (Table 2).

Perceived fatigue increased significantly over time in the clenching session ($P < .001$) with significantly higher levels of fatigue after clenching and after recovery compared to baseline ($P < .01$). No significant alterations occurred in the control session. Significantly higher levels of fatigue occurred after clenching ($P < .001$) and after recovery in the clenching session ($P < .01$) compared to the same time points in the control session.

No significant between-sex differences in perceived fatigue occurred at any time point in either session (Table 2).

Relative Recovery

Mean (SD) RR, based on a ^{14}C-lactate concentration of 29 ± 12% at baseline, was 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 changes occurred over time in either session.

5-HT and Glutamate

Interstitial 5-HT levels did not change over time in the clenching or the control session. No significant differences were observed between sessions at any time point (Fig 2). No significant sex differences were observed at any time point in any session (data not presented).

No significant alterations in muscle glutamate levels were observed over time in the clenching or the control session, and no significant differences occurred at any time point between the sessions (Fig 2). There were no significant sex differences at any time point in any session (data not presented). No significant differences were observed for 5-HT or glutamate at any time point between bruxers and non-bruxers (data not presented).

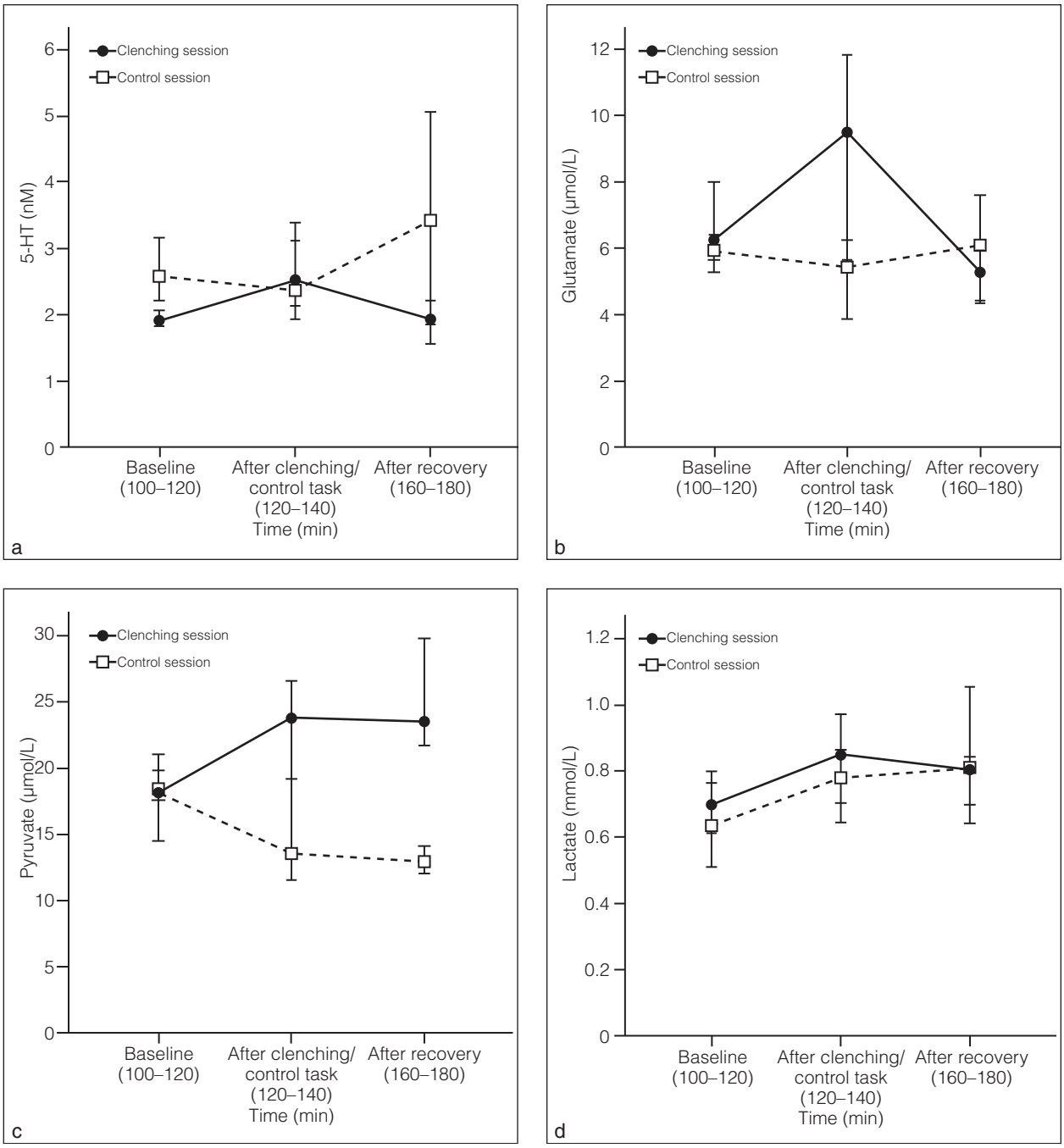

Fig 2 Interstitial concentrations (median, 75% and 25% percentile) of *(a)* 5-HT, *(b)* glutamate, *(c)* pyruvate, and *(d)* lactate in the masseter muscles of 30 healthy subjects in the clenching and the control sessions. No significant alterations over time were observed for these substances in either session. A comparison of time points in the clenching session with the same time points in the control session revealed no significant differences.

Pyruvate, Lactate, and Blood Flow

Muscle pyruvate levels were stable over time in the clenching and the control sessions, and there were no differences between sessions at any time point (Fig 2).

In females, pyruvate levels changed significantly over time in the clenching session ($P < .05$), with significantly higher levels of muscle pyruvate after the clenching task and after recovery compared to baseline ($P < .05$). No significant changes in muscle pyruvate occurred over time in the control session

Table 3 Median (Interquartile Range) Interstitial Concentration of Pyruvate (μmol/L) Measured at Baseline, After the Clenching and Control Task, and After Recovery

	Baseline		After clenching/control task		After recovery	
	Clenching	Control	Clenching	Control	Clenching	Control
Pyruvate (μmol/L)						
Females	17.7 (12)	19.6 (73.7)	21.2 (17.8)[a]	13.7 (30)	28.6 (19.8)[a]	15.4 (28.6)
Males	20.6 (66.8)	12.8 (15.5)	31.6 (62)	12.9 (15.5)	18.1 (30.4)	11.8 (14.4)

Significant difference compared with baseline in the same session ([a]$P < .01$).

or at any time point between sessions (Table 3). There were no significant changes in pyruvate levels in the males and no significant sex differences in any session at any time point (Table 3).

No significant changes in interstitial levels of lactate occurred over time in any session, and there were no significant differences between sessions at any time point (Fig 2). No significant sex differences occurred within or between sessions (data not presented).

Nutritive masseter muscle blood flow (out/inflow ratio, ^3H_2O) did not change over time in any sessions and there were no differences between sessions. There were no significant sex differences within or between sessions (data not presented).

Correlations

After Bonferroni correction for multiple testing, no significant correlations were identified between pain intensity after the tooth clenching and PPT, 5-HT, glutamate, pyruvate, lactate, cortisol, or β-estradiol.

Discussion

Although the experimental tooth-clenching model used in this study induced mechanical hyperalgesia and low levels of pain and fatigue, the levels of 5-HT, glutamate, and lactate were unaltered over time and between sessions in both sexes, except for muscle pyruvate, which was increased after clenching in females.

Patients with fibromyalgia and chronic work-related trapezius myalgia exhibit higher levels of interstitial pyruvate and lactate than healthy controls.[25,35] In healthy females, one study found that trapezius muscle pyruvate and lactate levels increased in response to low-force muscle exercise,[28] while another study found them to be unaffected by muscle exercise.[25] High levels of muscle pyruvate could be an indication of reduced tissue oxygenation,[27] in which case, pyruvate could be converted into lactate.[36] The role of lactate in muscle metabolism is highly complex, but if muscle lactate levels were associated with the intensity of muscle exercise, high levels of muscle lactate would indicate an ischemic condition in the muscle.[26,28,33] It has been suggested that even if tooth clenching increases intramuscular blood flow, this increase cannot meet the demands of the higher relative increase in muscle activity.[7] However, neither lactate nor pyruvate levels changed significantly in this study, except for pyruvate after the clenching task in females, and the blood flow did not change in response to the clenching task. Masseter muscle recovery during the 30-second resting intervals or a microdialysate sampling frequency that was insufficient to detect possible changes might explain the absence of significant changes. Future studies are needed to investigate the muscle metabolism in response to tooth clenching.

Intense muscle contraction could release algesic substances, leading to a nociceptor sensitization and a perception of pain.[37,38] Indeed, patients with myofascial TMD are reported to have higher interstitial levels of 5-HT and glutamate compared to healthy subjects,[22,23] and intramuscular injections of 5-HT and glutamate provoke pain, allodynia, and hyperalgesia in healthy subjects.[15,16] In the present study, experimental tooth clenching was used to investigate whether it could provoke a release of 5-HT and glutamate. Although the levels of pain and fatigue that occurred after clenching were low, they were significantly higher than in the control session. However, the masseter muscle levels of 5-HT and glutamate were unaltered in response to this experimental tooth-clenching task, and neither substance correlated with the VAS self-reports of pain intensity or fatigue. A study on healthy subjects and patients with trapezius myalgia found that the levels of interstitial 5-HT were not significantly altered over time in response to muscle exercise in either group,[24] which agrees with the present findings for 5-HT. However, regarding glutamate, the findings from this study contradict the results from a previous study in which increased glutamate levels were reported after muscle exercise in healthy subjects.[25] Methodological differences between studies may explain the divergent results for glutamate release. In

the previous studies referred to,[24,25] the participants performed a low-force muscle exercise continuously for 20 minutes with no relaxation periods. The tooth-clenching session in the present study comprised 20 bouts of clenching with 20 relaxation periods at 50% of MVCF, during which the masseter muscle could have recovered. A recent study reported that low-level clenching until exhaustion, but not high-intensity clenching, was associated with fatigue and reduced PPTs that persisted after 24 hours.[39] Therefore, it is possible that an experimental model using a continuous low-force clenching task could have led to a different result. Such a model would possibly lead to anerobic metabolism and perhaps to subsequent release of algesic substances. However, whether tooth clenching contributes to the higher muscle 5-HT and glutamate levels observed in patients with myofascial TMD is still unclear and warrants further investigation.

Thus it appears that the sensation of pain evoked in the tooth-clenching experimental model could not be explained by release of 5-HT or glutamate. Activation or sensitization of peripheral muscle nociceptors by other substances proposed to be involved in such processes might be one explanation; such substances include K^+ and H^+ ions,[40,41] prostaglandins,[42,43] cytokines,[44,45] and neuropeptides such as bradykinin[46] and calcitonin gene-related peptide.[47] These substances were not investigated in this study, and further research would be required to establish such a relationship and their role in muscle pain. Pilot data from the authors, however, indicate that interleukin (IL)-6 and IL-8 increase in response to experimental tooth clenching with similar protocol (personal communication).

Mechanical hyperalgesia occurred after tooth clenching, which agrees with the findings of a recent study,[48] but is in contrast to those of another.[39] The latter study observed that only low-intensity tooth-clenching exercises until exhaustion (7.5% and 10% of MVCF) were associated with a reduction in PPT. Two other studies found no association between low-intensity tooth-clenching and reduced PPT.[49,50] Differences in the tooth-clenching methodology used might explain the divergent results, as might the use of a microdialysis catheter inserted into the muscle. The catheter might have contributed to mechanical hyperalgesia, but because no hyperalgesia occurred in the control session, this is unlikely.

In concert with many other studies, the PPT in the masseter muscle was significantly higher in males compared to females.[49,51,52] No sex differences regarding the release of 5-HT, glutamate, or lactate were found, but in the females there was an increased release of pyruvate after clenching, which was not found in the males. This possible sex difference might indicate that women are more vulnerable to tissue oxygenation changes. It has been demonstrated that females have a higher percentage of type I muscle fibers and a lower percentage of type II muscle fibers than males in the masseter muscle.[53] During high-intensity muscle exercise, type II muscle fibers are recruited first.[54] Therefore, it could be speculated that these fibers will become exhausted more rapidly than in males, which could be a possible explanation for the higher pyruvate levels observed in females. The small number of subjects in the present study, however, means that the results must be interpreted with caution.

Variations in pain intensity during the menstrual cycle have been observed in females with myofascial TMD.[55] An interaction between estrogen, cortisol, and the central release of 5-HT has also been observed.[19–21] The present study did not confirm an association between estrogen and cortisol levels and a peripheral release of 5-HT or glutamate. Neither was a correlation observed between blood-estradiol levels and pain intensity or PPT. Although estrogen may affect pain and PPT levels, most studies have found no correlation between sex hormone levels and pain/PPT levels, similar to the present findings.[56,57]

Psychological stress is a possible risk factor for myofascial TMD.[6] Prolonged release of cortisol could be associated with hyperalgesia.[58,59] In the present study, the levels of salivary cortisol were not significantly correlated with levels of muscle 5-HT, pain intensity, or PPT, possibly because the participants were healthy and did not suffer from myofascial TMD.

Some of the microdialysate samples did not have detectable levels of any of the substances, which might have affected the results. Another limitation is that the phases of the menstrual cycle were not controlled in females. Sex hormone levels affect pain levels,[55] but the magnitude of this effect is probably low.[56,57] The women in this study were most likely in different phases of the cycle, which would negate any effects. Lack of variations in estradiol between sessions supports this. One strength in the present study is that the participants were questioned about awareness of awake and sleep bruxism; a post-hoc analysis confirmed no association between self-reported bruxism and release of 5-HT or glutamate. Another strength is that this is, to the authors' knowledge, the first study to investigate the peripheral release of 5-HT, glutamate, lactate, and pyruvate in the human masseter muscle with the microdialysis technique after experimental tooth clenching.

In conclusion, although low levels of pain, mechanical hyperalgesia, and fatigue developed after experimental tooth clenching in the present study, the clenching effort did not provoke a release of 5-HT, glutamate, pyruvate, or lactate. No sex differences for these substances were observed. Future research using modified clenching models and also focusing on other algesic substances is needed to elucidate the peripheral release of algesic substances in the masseter muscle in response to tooth clenching.

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References

1. Breivik H, Collett B, Ventafridda V, Cohen R, Gallacher D. Survey of chronic pain in Europe: Prevalence, impact on daily life, and treatment. Eur J Pain 2006;10:287–333.
2. Drangsholt M, LeResche L. Temporomandibular disorder pain. In: Crombie IK, Croft PR, Linton SJ, LeResche L, Von Korff M (eds). Epidemiology of Pain. Seattle: IASP Press, 1999:203–233.
3. Dworkin SF, Huggins KH, LeResche L, et al. Epidemiology of signs and symptoms in temporomandibular disorders: Clinical signs in cases and controls. J Am Dent Assoc 1990;120:273–281.
4. Dworkin SF, LeResche L. Research diagnostic criteria for temporomandibular disorders: Review, criteria, examinations and specifications, critique. J Craniomandib Disord 1992;6:301–355.
5. Sessle BJ. Peripheral and central mechanisms of orofacial pain and their clinical correlates. Minerva Anestesiol 2005;71:117–136.
6. Velly AM, Gornitsky M, Philippe P. Contributing factors to chronic myofascial pain: A case-control study. Pain 2003;104:491–499.
7. Monteiro AA, Kopp S. Estimation of blood flow by 133Xe clearance in human masseter muscle during rest, endurance of isometric contraction, and recovery. Arch Oral Biol 1988;33:561–565.
8. Mense S. Nociception from skeletal muscle in relation to clinical muscle pain. Pain 1993;54:241–289.
9. Ernberg M, Lundeberg T, Kopp S. Effect of propranolol and granisetron on experimentally induced pain and allodynia/hyperalgesia by intramuscular injection of serotonin into the human masseter muscle. Pain 2000;84:339–346.
10. Cairns BE, Svensson P, Wang K, et al. Activation of peripheral NMDA receptors contributes to human pain and rat afferent discharges evoked by injection of glutamate into the masseter muscle. J Neurophysiol 2003;90:2098–2105.
11. Zeitz KP, Guy N, Malmberg AB, et al. The 5-HT3 subtype of serotonin receptor contributes to nociceptive processing via a novel subset of myelinated and unmyelinated nociceptors. J Neurosci 2002;22:1010–1019.
12. Ro JY, Capra NF, Lee JS, Masri R, Chun YH. Hypertonic saline-induced muscle nociception and c-fos activation are partially mediated by peripheral NMDA receptors. Eur J Pain 2007;11:398–405.
13. Lam DK, Sessle BJ, Hu JW. Glutamate and capsaicin effects on trigeminal nociception I: Activation and peripheral sensitization of deep craniofacial nociceptive afferents. Brain Res 2009;1251:130–139.
14. Lam DK, Sessle BJ, Hu JW. Glutamate and capsaicin effects on trigeminal nociception II: Activation and central sensitization in brainstem neurons with deep craniofacial afferent input. Brain Res 2009;1253:48–59.
15. Castrillon EE, Cairns BE, Ernberg M, et al. Glutamate-evoked jaw muscle pain as a model of persistent myofascial TMD pain? Arch Oral Biol 2008;53:666–676.
16. Ernberg M, Lundeberg T, Kopp S. Pain and allodynia/hyperalgesia induced by intramuscular injection of serotonin in patients with fibromyalgia and healthy individuals. Pain 2000;85:31–39.
17. Kuttila M, Niemi PM, Kuttila S, Alanen P, Le Bell Y. TMD treatment need in relation to age, gender, stress, and diagnostic subgroup. J Orofac Pain 1998;12:67–74.
18. Korszun A, Young EA, Singer K, Carlson NE, Brown MB, Crofford L. Basal circadian cortisol secretion in women with temporomandibular disorders. J Dent Res 2002;81:279–283.
19. van Praag HM. Faulty cortisol/serotonin interplay. Psychopathological and biological characterisation of a new, hypothetical depression subtype (SeCA depression). Psychiatry Res 1996;65:143–157.
20. Bethea CL, Lu NZ, Gundlah C, Streicher JM. Diverse actions of ovarian steroids in the serotonin neural system. Front Neuroendocrinol 2002;23:41–100.
21. Mora F, Segovia G, Del Arco A, de Blas M, Garrido P. Stress, neurotransmitters, corticosterone and body-brain integration. Brain Res 2012;1476:71–85.
22. Ernberg M, Hedenberg-Magnusson B, Alstergren P, Kopp S. The level of serotonin in the superficial masseter muscle in relation to local pain and allodynia. Life Sci 1999;65:313–325.
23. Castrillon EE, Ernberg M, Cairns BE, et al. Interstitial glutamate concentration is elevated in the masseter muscle of myofascial temporomandibular disorder patients. J Orofac Pain 2010;24:350–360.
24. Ghafouri B, Larsson BK, Sjors A, Leandersson P, Gerdle BU. Interstitial concentration of serotonin is increased in myalgic human trapezius muscle during rest, repetitive work and mental stress—An in vivo microdialysis study. Scand J Clin Lab Invest 2010;70:478–486.
25. Rosendal L, Larsson B, Kristiansen J, et al. Increase in muscle nociceptive substances and anaerobic metabolism in patients with trapezius myalgia: Microdialysis in rest and during exercise. Pain 2004;112:324–334.
26. Setala LP, Korvenoja EM, Harma MA, Alhava EM, Uusaro AV, Tenhunen JJ. Glucose, lactate, and pyruvate response in an experimental model of microvascular flap ischemia and reperfusion: A microdialysis study. Microsurgery 2004;24:223–231.
27. Goda N, Kanai M. Hypoxia-inducible factors and their roles in energy metabolism. Int J Hematol 2012;95:457–463.
28. Flodgren GM, Hellstrom FB, Fahlstrom M, Crenshaw AG. Effects of 30 versus 60 min of low-load work on intramuscular lactate, pyruvate, glutamate, prostaglandin E(2) and oxygenation in the trapezius muscle of healthy females. Eur J Appl Physiol 2006;97:557–565.

29. Hedenberg-Magnusson B, Brodda Jansen G, Ernberg M, Kopp S. Effects of isometric contraction on intramuscular level of neuropeptide Y and local pain perception. Acta Odontol Scand 2006;64:360–367.
30. List T, Helkimo M, Falk G. Reliability and validity of a pressure threshold meter in recording tenderness in the masseter muscle and the anterior temporalis muscle. Cranio 1989;7:223–229.
31. Ernberg M, Castrillon EE, Ghafouri B, et al. Experimental myalgia induced by repeated infusion of acidic saline into the human masseter muscle does not cause the release of algesic substances. Eur J Pain 2013;17:539–550.
32. Lonnroth P, Jansson PA, Smith U. A microdialysis method allowing characterization of intercellular water space in humans. Am J Physiol 1987;253(2 Pt 1):E228–E231.
33. Larsson B, Rosendal L, Kristiansen J, et al. Responses of algesic and metabolic substances to 8 h of repetitive manual work in myalgic human trapezius muscle. Pain 2008; 140:479–490.
34. Gerdle B, Lemming D, Kristiansen J, Larsson B, Peolsson M, Rosendal L. Biochemical alterations in the trapezius muscle of patients with chronic whiplash associated disorders (WAD)—A microdialysis study. Eur J Pain 2008;12:82–93.
35. Gerdle B, Soderberg K, Salvador Puigvert L, Rosendal L, Larsson B. Increased interstitial concentrations of pyruvate and lactate in the trapezius muscle of patients with fibromyalgia: A microdialysis study. J Rehabil Med 2010;42: 679–687.
36. Philp A, Macdonald AL, Watt PW. Lactate—A signal coordinating cell and systemic function. J Exp Biol 2005;208: 4561–4575.
37. MacLean DA, LaNoue KF, Gray KS, Sinoway LI. Effects of hindlimb contraction on pressor and muscle interstitial metabolite responses in the cat. J Appl Physiol 1998;85: 1583–1592.
38. Sjogaard G, Lundberg U, Kadefors R. The role of muscle activity and mental load in the development of pain and degenerative processes at the muscle cell level during computer work. Eur J Appl Physiol 2000;83:99–105.
39. Farella M, Soneda K, Vilmann A, Thomsen CE, Bakke M. Jaw muscle soreness after tooth-clenching depends on force level. J Dent Res 2010;89:717–721.
40. Clarkson PM, Sayers SP. Etiology of exercise-induced muscle damage. Can J Appl Physiol 1999;24:234–248.
41. Muller M, Schmid R, Nieszpaur-Los M, et al. Key metabolite kinetics in human skeletal muscle during ischaemia and reperfusion: Measurement by microdialysis. Eur J Clin Invest 1995;25:601–607.
42. Graven-Nielsen T, McArdle A, Phoenix J, et al. In vivo model of muscle pain: Quantification of intramuscular chemical, electrical, and pressure changes associated with saline-induced muscle pain in humans. Pain 1997;69:137–143.
43. Smith LL, Fulmer MG, Holbert D, et al. The impact of a repeated bout of eccentric exercise on muscular strength, muscle soreness and creatine kinase. Br J Sports Med 1994;28: 267–271.
44. Nosaka K, Clarkson PM. Changes in indicators of inflammation after eccentric exercise of the elbow flexors. Med Sci Sports Exerc 1996;28:953–961.
45. Pedersen BK, Ostrowski K, Rohde T, Bruunsgaard H. The cytokine response to strenuous exercise. Can J Physiol Pharmacol 1998;76:505–511.
46. Babenko V, Graven-Nielsen T, Svensson P, Drewes AM, Jensen TS, Arendt-Nielsen L. Experimental human muscle pain and muscular hyperalgesia induced by combinations of serotonin and bradykinin. Pain 1999;82:1–8.
47. Kehl LJ, Trempe TM, Hargreaves KM. A new animal model for assessing mechanisms and management of muscle hyperalgesia. Pain 2000;85:333–343.
48. Dawson A, List T, Ernberg M, Svensson P. Assessment of proprioceptive allodynia after tooth-clenching exercises. J Orofac Pain 2012;26:39–48.
49. Okayasu I, Oi K, De Laat A. The effect of tooth clenching on the sensory and pain perception in the oro-facial region of symptom-free men and women J Oral Rehabil 2009; 36:476–482.
50. Svensson P, Burgaard A, Schlosser S. Fatigue and pain in human jaw muscles during a sustained, low-intensity clenching task. Arch Oral Biol 2001;46:773–777.
51. Komiyama O, Kawara M, De Laat A. Ethnic differences regarding tactile and pain thresholds in the trigeminal region. J Pain 2007;8:363–369.
52. Komiyama O, De Laat A. Tactile and pain thresholds in the intra- and extra-oral regions of symptom-free subjects. Pain 2005;115:308–315.
53. Tuxen A, Bakke M, Pinholt EM. Comparative data from young men and women on masseter muscle fibres, function and facial morphology. Arch Oral Biol 1999;44:509–518.
54. Park JH, Brown RL, Park CR, et al. Functional pools of oxidative and glycolytic fibers in human muscle observed by 31P magnetic resonance spectroscopy during exercise. Proc Natl Acad Sci U S A 1987;84:8976–8980.
55. LeResche L, Mancl L, Sherman JJ, Gandara B, Dworkin SF. Changes in temporomandibular pain and other symptoms across the menstrual cycle. Pain 2003;106:253–261.
56. Stening KD, Berg G, Hammar M, et al. Influence of estrogen levels on thermal perception, pain thresholds, and pain tolerance: Studies on women undergoing in vitro fertilization. J Pain 2012;13:459–466.
57. Vignolo V, Vedolin GM, de Araujo Cdos R, Rodrigues Conti PC. Influence of the menstrual cycle on the pressure pain threshold of masticatory muscles in patients with masticatory myofascial pain. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;105:308–315.
58. Dufton LM, Konik B, Colletti R, et al. Effects of stress on pain threshold and tolerance in children with recurrent abdominal pain. Pain 2008;136:38–43.
59. Imbe H, Iwai-Liao Y, Senba E. Stress-induced hyperalgesia: Animal models and putative mechanisms. Front Biosci 2006;11:2179–2192.