# Pain, Allodynia, and Serum Serotonin Level in Orofacial Pain of Muscular Origin

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**Aims:** *This study was conducted to investigate the serum level of serotonin (S-5-HT) in patients with temporomandibular disorders (TMD) of muscular origin, ie, localized myalgia, and to compare it to that found in healthy individuals and patients with fibromyalgia. A second aim was to investigate the association between S-5-HT and pain parameters.* **Methods:** *Twenty patients with localized myalgia participated in the study. Twenty age- and gender-matched healthy individuals and twenty patients with fibromyalgia served as controls. The participants were examined clinically as to the condition of the temporomandibular region and S-5-HT.* **Results:** *The levels of S-5-HT did not differ significantly between the groups. However, in patients with localized myalgia there was a negative correlation between S-5-HT and tenderness of the temporomandibular muscles.* **Conclusion:** *The results of this study indicate that allodynia of orofacial muscles in patients with TMD is significantly related to S-5-HT concentration.*
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**Key words:** fibromyalgia, allodynia, local myalgia, serotonin, temporomandibular disorders

Pain in the orofacial muscles is a common reason for patients to seek treatment at pain clinics. Many of these patients suffer from temporomandibular disorders (TMD), while some patients with orofacial muscle pain suffer from fibromyalgia. Temporomandibular disorders of muscular origin are characterized by localized myalgia and tenderness to palpation, with or without referral of pain to other regions.[1,2] The etiology and pathophysiology is unclear, but it has for a long time been assumed to develop after overloading of muscles because of, eg, nocturnal bruxism and ensuing muscle ischemia.[3,4] Sleep disturbance, depression, and headache are commonly associated with chronic localized myalgia.[5,6] Fibromyalgia is characterized by generalized muscle pain and tenderness, muscle stiffness, and fatigue.[7] Sleep disturbances, headache, and depression are also frequent symptoms in fibromyalgia. For research purposes, the criteria of the American College of Rheumatology for classifying fibromyalgia are the most often used.[8] These criteria include widespread pain for more than 3 months and tenderness to palpation on at least 11 of 18 specific bilateral points.

Table 1 Age, Gender, and Duration of Local and General Symptoms in Study Participants

| | Patients with localized myalgia | Patients with fibromyalgia | Healthy individuals |
|---|---|---|---|
| Age (y) (mean ± SEM) | 46 ± 4 | 49 ± 3 | 44 ± 2 |
| Gender (male/female) | 4/16 | 0/20 | 4/16 |
| Duration of local symptoms (y) (mean ± SEM) | 7 ± 1 | 9 ± 2 | — |
| Duration of general symptoms (y) (mean ± SEM) | — | 11 ± 1 | — |

SEM = standard error of the mean.

There is evidence that serotonin (5-HT) is involved in the pathophysiology of chronic myalgia. Moldofsky and Warsh[9] reported an inverse association between plasma tryptophan (a precursor to 5-HT) levels and the level of pain reported by patients with fibromyalgia. Yunus et al[10] found a reduced level of plasma tryptophan and a decreased transport ratio of tryptophan across the blood-brain barrier in fibromyalgia. Russell et al[11] found that 5-hydroxyindoleacetic acid, the metabolite of 5-HT, was decreased in the cerebrospinal fluid of patients with fibromyalgia. Furthermore, the serum level of 5-HT (S-5-HT) has been reported to be lower in patients with fibromyalgia than in healthy control patients.[12] Recently, Wolfe et al[7] investigated the level of S-5-HT in a population survey. In agreement with previous studies, they found a lower level of S-5-HT in patients with fibromyalgia compared to pain-free individuals. They also found a positive correlation between the level of S-5-HT and tender point count as well as depression score, which contradicts previous findings.[9,11,12] This also contradicts reports that 5-HT re-uptake inhibitors have been effective in alleviating pain and tenderness in some patients with fibromyalgia and that they can have a positive effect on the patient's well-being.[6,13] While the role of blood 5-HT in fibromyalgia and tension-type headache[14] as well as in rheumatoid arthritis[12,15] has been investigated frequently, its role in TMD of muscular origin remains unknown. Only a few studies seem to have addressed this subject. Among them, the study of Sharav et al,[16] which investigated the effect of amitriptyline hydrochloride on chronic facial pain of musculoskeletal origin, found that the drug was more effective than a placebo in reducing pain, and that it did so independently of its antidepressant action. Wolfe et al[7] could not detect any difference in S-5-HT between individuals with regional musculoskeletal pain and pain-free individuals.

To the knowledge of the authors, there are no studies of the level of S-5-HT in patients with TMD of muscular origin. The first aim of this study was therefore to investigate the level of S-5-HT in patients with chronic localized myalgia of the temporomandibular region and to compare it to that found in healthy individuals and patients with fibromyalgia. The second aim was to investigate the association between the level of S-5-HT and pain parameters.

## Materials and Methods

### Subjects

Twenty consecutive patients with localized myalgia (four men and sixteen women) and 20 consecutive patients with fibromyalgia (all women) who had been referred to the department of Clinical Oral Physiology because of orofacial pain of muscular origin participated in the study (Table 1). The authors intended to match the patients with fibromyalgia to the patients with localized myalgia regarding age and gender but were unable to find any men with fibromyalgia. The patients were first given a brief clinical examination to determine whether they fulfilled the inclusion criteria. These were pain from the temporomandibular region for at least 3 months and tenderness of the orofacial muscles to digital palpation. For the patients included in the fibromyalgia group, fibromyalgia was diagnosed according to the criteria of the American College of Rheumatology.[8] Exclusion criteria were general inflammatory connective tissue disease (eg, rheumatoid arthritis) or symptoms that could be related to disease in other orofacial areas (eg, temporomandibular joints, toothache,

neuralgia). Four of the patients with fibromyalgia were taking antidepressants: one was taking citalopram, one was taking clomipramine, and two were taking amitriptyline hydrochloride.

A group of 20 healthy individuals who participated on a voluntary basis were also included in the study. They were age- and gender-matched to the patients with localized myalgia and had no pain in the temporomandibular region.

The methods and selection of patients were approved by the local ethical committee at Huddinge Hospital, Karolinska Institutet, Stockholm (151/93). All individuals gave their verbal consent.

## Methods

*Assessment of Pain.* A 10-cm visual analogue scale (VAS) with endpoints marked by "no pain" and "worst pain ever experienced" was used to assess the greatest degree of pain from the orofacial region experienced during the last week. The patients with fibromyalgia were asked to assess their general pain on a VAS in a similar way and were interrogated about pain experienced in certain musculoskeletal body regions. These regions were: neck, arm, elbow, buttocks, knee, and ankle. The sum of affirmative answers (maximum of 6) was referred to as the number of painful body regions.

*Clinical Examination.* Routine methods of clinical examination of the temporomandibular system were used.[17] The degree of tenderness to digital palpation was assessed over the superficial masseter, anterior temporal, temporal tendon insertion, lateral pterygoid, medial pterygoid, and trapezius. A 4-grade scale, modified after Russell et al[18] was used (0 = no tenderness, 1 = mild tenderness, 2 = moderate tenderness with a pain reflex, and 3 = marked tenderness with a defensive withdrawal reflex). The sum of tenderness grades, with a maximum of 36, was calculated for each patient and is referred to as the tender point index (TPI).[18] The pressure pain threshold (PPT) and pressure pain tolerance level (PPTL) were assessed (kPa) with an algometer (Pain Diagnostics and Thermography) over the most tender point on the 2 superficial masseter muscles. These muscles were chosen since they are easily examined and are often tender upon digital palpation in patients with TMD. The algometer had a blunt rubber recording tip 10 mm in diameter. The pressure rate used was approximately 50 kPa/s. The PPT was defined as the level of pressure at which the patient reported that the sensation of pressure changed to pain. At a subsequent recording the PPTL was defined as the level of pressure at which the patient reported that the pain became intolerable. The sum of the PPT and PPTL values from the right and left side was calculated for each patient and referred to as $PPT_{sum}$ and $PPTL_{sum}$.

*Blood Examination.* A sample of 28 mL of venous blood was collected from each patient. The erythrocyte sedimentation rate, C-reactive protein, hemoglobin, rheumatoid factor, and antinuclear antibodies were analyzed. This was done partly to detect any undiscovered systemic connective tissue disease. The thrombocyte particle concentration was also analyzed, since platelets are the main source of 5-HT in blood serum and therefore could be expected to influence the level of S-5-HT. Another sample of 2 mL was collected from each participant for analysis of the level of S-5-HT. This blood sample was left at room temperature for 1 hour to coagulate and then was cold centrifuged (4°C, 1700 G) for 30 minutes. Approximately 200 µL of the supernatant was then pipetted into a polystyrene tube and kept frozen at –22°C until analysis. To exclude any risk of interference with the analysis of 5-HT, patients were asked to avoid tryptophan-rich food (eg, banana, pineapple, tomato, and chocolate) for 24 hours before the examination. Because of the diurnal variation of S-5-HT,[19] all blood samples were collected in the morning.

*Biochemical Analysis.* The level of S-5-HT was analyzed by a commercially available competitive enzyme immunoassay kit (No. 0642, Immunotech International). The kit has a sensitivity of 0.09 ng/mL and is applicable at concentrations between 0.28 and 35.2 ng/mL. According to the manufacturer, the intraassay coefficient of variation varies between 7.9% and 9.4%, and the interassay coefficient of variation varies between 6.2% and 9.9%.

## Statistics

The Kolmogorov-Smirnov test for continuous variables was used to test the normality of the variables. One-way analysis of variance (ANOVA) with the Tukey test for pairwise multiple comparison as an ad hoc test was used to test the significance of the differences between groups regarding normally distributed variables. One-way ANOVA on ranks with Dunn's method for pairwise multiple comparison as an ad hoc test or the Mann-Whitney U test was used to test the significance of differences between groups regarding nonnormally distributed variables or variables on an ordinal scale. Pearson's product-moment correlation analysis was used to test the relationship between

S-5-HT and clinical variables except for data on an ordinal scale (VAS and TPI), which were tested with Spearman's ranked correlation test. All tests were 2-tailed and the significance level was set at $P < 0.05$.

## Results

### Serum Level of 5-HT

The levels of S-5-HT are shown in Fig 1. In the group with localized myalgia the S-5-HT ranged between 14 and 299 ng/mL, in the group with fibromyalgia it ranged between 6 and 306 ng/mL, and in the group of healthy individuals it ranged between 18 and 338 ng/mL. There was no significant difference in S-5-HT level between the groups. When the 4 fibromyalgia patients who were under medication with antidepressant drugs were excluded, still no significant difference was found between groups.

### Assessment of Pain

The VAS score in the group with localized myalgia (median and interquartile range) was 6 ± 2; in the group with fibromyalgia it was 5 ± 3. The difference between these patient groups was significant ($P < 0.05$, Mann-Whitney U test). The general VAS in the group with fibromyalgia was 6 ± 4, and the number of painful body regions was 5 ± 1.

### Assessment of Tenderness

The assessments of muscle tenderness are shown in Table 2. The TPI was similar in both patient groups and lower than in the group with healthy individuals. The difference between groups was significant ($P < 0.001$). Multiple comparison according to Dunn's test showed that both patient groups differed from the healthy individuals ($P < 0.05$) but there was no difference between the patient groups.

Both PPT and PPTL were lower in the patient groups than in the healthy individuals, with the lowest values found in the patients with fibromyalgia. The 3 groups differed regarding $PPTL_{sum}$ ($P < 0.001$), but not as regards $PPT_{sum}$. Multiple comparison according to Tukey showed that there were significant differences between all 3 groups regarding $PPTL_{sum}$ ($P < 0.05$).

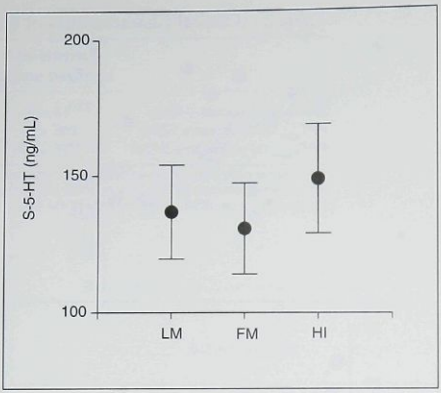


**Fig 1** Plot shows the mean serum level (ng/mL, ± SEM) of serotonin (S-5-HT) in patients with localized myalgia and fibromyalgia of the temporomandibular region and in healthy individuals. There were 20 subjects in each group. The mean S-5-HT (± SEM) was 137 ± 17 ng/mL in the patients with localized myalgia, 131 ± 17 ng/mL in the patients with fibromyalgia, and 149 ± 20 ng/mL in the healthy individuals. LM = patients with localized myalgia; FM = patients with fibromyalgia; HI = healthy individuals.

### Blood Variables

Two patients with localized myalgia had slightly increased erythrocyte sedimentation rates (47 and 40 mm/h, respectively). One of the patients was a 72-year-old female with a present inflammation of the urinary tract; the other was a 56-year-old male who had had otitis. It is unlikely that their symptoms from temporomandibular muscles were a result of these infections, since the pain was present before the onset of the infections. Furthermore, their symptoms did not increase during infection and persisted after they were cured. A 45-year-old female patient with localized myalgia tested positive for antinuclear antibodies (ANA) but had no rheumatic disease. The thrombocyte particle concentration was within the normal range for all patients in both patient groups and showed no correlation to S-5-HT.

### Correlations

Several significant correlations were found. In the group with localized myalgia there was a negative correlation between S-5-HT and TPI ($r_s = -0.57$,

Table 2 Clinical Characteristics of Study Participants

| | Patients with localized myalgia | Patients with fibromyalgia | Healthy individuals |
|---|---|---|---|
| TPI (0–36) (median ± IQR) | 12 ± 8 | 13 ± 7 | 2 ± 3 |
| $PPT_{sum}$ (kPa) (mean ± SEM) | 286 ± 31 | 241 ± 20 | 334 ± 27 |
| $PPTL_{sum}$ (kPa) (mean ± SEM) | 575 ± 37 | 421 ± 31 | 727 ± 46 |

TPI = tender point index of temporomandibular muscles; $PPT_{sum}$ = sum of the pressure pain threshold of the right and left masseter muscles; $PPTL_{sum}$ = sum of the pressure pain tolerance level of the right and left masseter muscle; IQR = interquartile range; SEM = standard error of the mean.

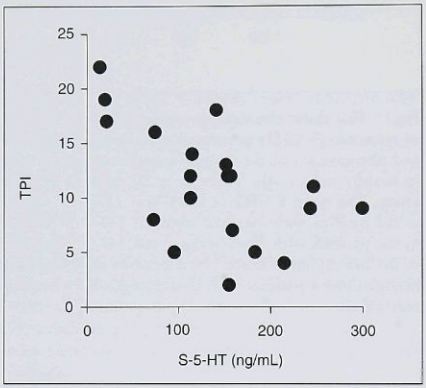


Fig 2 Scatter plot shows the correlation between the serum level (ng/mL) of serotonin (S-5-HT) and the tender point index (TPI) of temporomandibular muscles in patients with localized myalgia of the temporomandibular region ($r_s$ = –0.57, n = 20, $P < 0.01$).

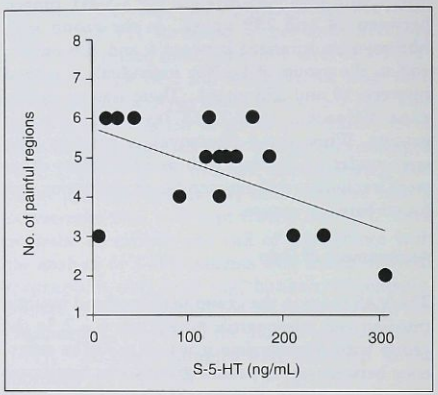


Fig 3 Scatter plot shows the correlation between the serum level (ng/mL) of serotonin (S-5-HT) and the number of painful body regions in patients with fibromyalgia of the temporomandibular region ($r$ = –0.55, n = 16, $P < 0.05$).

n = 20, $P < 0.01$; Fig 2). In the group with fibromyalgia there was a negative correlation between S-5-HT and the number of painful musculoskeletal body regions ($r$ = –0.55, n = 16, $P < 0.05$; Fig 3) and a positive correlation between S-5-HT and $PPTL_{sum}$ ($r$ = 0.49, n = 19, $P < 0.05$; Fig 4).

## Discussion

The main result of our study is the finding of an association between low S-5-HT level and local allodynia of temporomandibular muscles in patients with chronic TMD of muscular origin. To our knowledge, this has not been reported previously. In the patients with localized myalgia there was a significant correlation between low S-5-HT and high TPI of temporomandibular muscles, which indicates that S-5-HT has a considerable influence on tenderness to digital palpation or local allodynia in this patient group. This remarkable finding indicates that central mechanisms are involved in the modulation of allodynia in chronic TMD of muscular origin. It also supports the theory that pathologic alterations of the antinociceptive system may lead to allodynia.[20] Furthermore, the finding may explain why some patients with localized myalgia report improvement in their condition when using serotonin reuptake inhibitors.[16] This result, however, contradicts the finding[15] of an association between high S-5-HT and allodynia of the temporomandibular joint in patients with

seropositive rheumatoid arthritis. The reason for this difference is unclear, but might be due to the pathophysiologic differences between these 2 patient categories as well as between joints and muscles.

In the patients with fibromyalgia, significant correlations were found between low S-5-HT and a high number of painful musculoskeletal body regions, and between low S-5-HT and low PPTL. These findings concur with the previous studies by Moldofsky and Warsh,[9] Russell et al,[11] and Stratz et al,[12] but contradict the study by Wolfe et al[7] regarding tenderness. It is difficult to explain these conflicting results. However, the study by Wolfe et al[7] was performed in an epidemiologic population survey, while our study was performed on patients attending treatment. There is reason to believe that the intensity and character of pain differs between these populations.

Our study showed no significant difference in S-5-HT between the groups. In all 3 groups the variation in S-5-HT was large, and we found localized myalgia and fibromyalgia patients with high S-5-HT levels and healthy individuals with low S-5-HT. Regarding localized myalgia, our results in this particular aspect agree with those of Wolfe et al,[7] who found no difference in S-5-HT between patients with regional chronic musculoskeletal pain and pain-free individuals in a population survey. On the other hand, our results disagree with many reports of a reduced blood serotonin level in patients with fibromyalgia.[7,10–12] The levels of S-5-HT in our study are also similar to those found by Alstergren et al[15] in a group of patients with rheumatoid arthritis.

Since low S-5-HT may be a result of fewer platelets per volume of blood, we also measured the thrombocyte particle concentration in the patients. In both patient groups the values were within the normal range, and no correlation to the S-5-HT was found. Consequently, it is unlikely that this factor influenced our results or that the thrombocyte particle concentration influences S-5-HT. Four patients with fibromyalgia were taking antidepressant medication. Therefore, we also investigated whether a significant difference in S-5-HT between groups could be found when these patients were excluded. However, no significant difference was found.

It is puzzling that we found no difference in S-5-HT between groups but at the same time found significant correlations in the patient groups between allodynia/hyperalgesia of the temporomandibular muscles and low S-5-HT. The patients with the lowest S-5-HT thus showed the greatest degree of muscle tenderness. One explanation could be that there is an increased binding of 5-HT to receptors for pain and allodynia/hyperalgesia in patients with chronic myalgia and that this condition is associated with low S-5-HT.

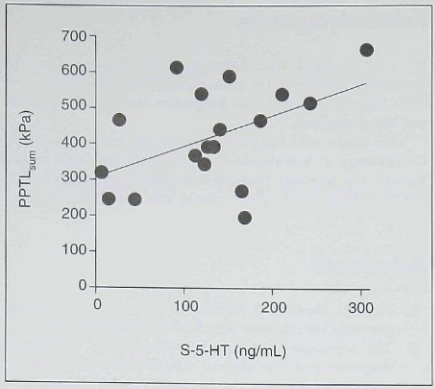


Fig 4 Scatter plot shows the correlation between the serum level (ng/mL) of serotonin (S-5-HT) and the pressure pain tolerance level ($PPTL_{sum}$; in kPa) in patients with fibromyalgia of the temporomandibular region ($r$ = 0.49, n = 19, $P < 0.05$).

It was our intention to match the healthy individuals and the patients with fibromyalgia to the patients with localized myalgia regarding both age and gender, but we were unable to find any men with fibromyalgia. There was, however, no significant difference in age and duration of local symptoms between the 2 patient groups. We consider the difference in local pain (VAS) between the patient groups to be clinically insignificant and we therefore consider the groups comparable.

The PPT and PPTL of the masseter muscles were lower in the patients with fibromyalgia than in the patients with localized myalgia. Even though the difference regarding the PPT was only close to statistical significance, the finding is in agreement with previous studies.[6,21,22] These findings may reflect an increased sensitivity to mechanical stimuli in general in patients with fibromyalgia, as has been suggested previously.[6,21]

The results of this study indicate that allodynia of orofacial muscles in chronic TMD is significantly related to S-5-HT concentration.

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## References

1. Han SC, Harrison P. Myofascial pain syndrome and trigger-point management. Reg Anesth 1997;22:89–101.
2. The American Academy of Orofacial Pain. Differential diagnosis and management considerations of temporomandibular disorders. In: Okeson JP (ed). Orofacial Pain. Guidelines for Assessment, Diagnosis and Management. Chicago: Quintessence, 1996:137–141.
3. Foreman PA. Temporomandibular joint and myofascial pain dysfunction—Some current concepts. Part 1: Diagnosis. NZ Dent J 1985;81:47–52.
4. Larsson SE, Bodegard L, Henriksson KG, Oberg PA. Chronic trapezius myalgia. Morphology and blood flow studied in 17 patients. Acta Orthop Scand 1990;61: 394–398.
5. Harness DM, Donlon WC, Eversole LR. Comparison of clinical characteristics in myogenic, TMJ internal derangement and atypical facial pain patients. Clin J Pain 1990;6: 4–17.
6. Scudds RA, McCain GA, Rollman GB, Harth M. Improvements in pain responsiveness in patients with fibrositis after successful treatment with amitriptyline. J Rheumatol 1989;19(suppl):98–103.
7. Wolfe F, Russell IJ, Vipraio G, Ross K, Anderson J. Serotonin levels, pain threshold, and fibromyalgia symptoms in the general population. J Rheumatol 1997;24:555–559.
8. Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DL, et al. The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. Arthritis Rheum 1990;33:160–172.
9. Moldofsky H, Warsh JJ. Plasma tryptophan and musculoskeletal pain in non-articular rheumatism ("fibrositis syndrome"). Pain 1978;5:65–71.
10. Yunus MB, Dailey JW, Aldag JC, Masi AT, Jobe PC. Plasma tryptophan and other amino acids in primary fibromyalgia: A controlled study. J Rheumatol 1992;19:90–94.
11. Russell IJ, Vaeroy H, Javors M, Nyberg F. Cerebrospinal fluid biogenic amine metabolites in fibromyalgia/fibrositis syndrome and rheumatoid arthritis. Arthritis Rheum 1992;35:550–556.
12. Stratz T, Samborski W, Hrycaj P, Pap T, Mackiewicz S, Mennet P, Muller W. Die serotoninkonzentration im serum bei patienten mit generalisierter tendomyopathie (fibromyalgie) und chronischer polyarthritis. Med Klin 1993;88:458–462.
13. Carette S, McCain GA, Bell DA, Fam AG. Evaluation of amitriptyline in primary fibrositis. A double-blind, placebo-controlled study. Arthritis Rheum 1986;29:655–659.
14. Marcus DA. Serotonin and its role in headache pathogenesis and treatment. Clin J Pain 1993;9:159–167.
15. Alstergren P, Ernberg M, Kopp S, Lundeberg T, Theodorsson E. TMJ pain in relation to circulating neuropeptide Y, serotonin, and interleukin-1ß in rheumatoid arthritis. J Orofac Pain 1999;13:49–55.
16. Sharav Y, Singer E, Schmidt E, Dionne RA, Dubner R. The analgesic effect of amitriptyline on chronic facial pain. Pain 1987;31:199–209.
17. Krough-Poulsen W. Management of the occlusion of teeth: Examination, diagnosis, treatment. In: Schwartz NL, Chayes CM (eds). Facial Pain and Mandibular Dysfunction. Philadelphia: WB Saunders, 1972:252–258.
18. Russell IJ, Vipraio GA, Morgan WW, Bowden CL. Is there a metabolic basis for the fibrositis syndrome? Am J Med 1986;81:50–54.
19. Candito M, Souetre E, Iordache A, Pringuey D, Ardisson JL, Chambon P, Darcourt G. Diurnal variation in total plasma tryptophan in controls and in depression. J Psychiatr Res 1990;24:227–230.
20. Mense S. Nociception from skeletal muscle in relation to clinical muscle pain. Pain 1993;54:241–289.
21. Kosek E, Ekholm J, Hansson P. Sensory dysfunction in fibromyalgia patients with implications for pathogenic mechanisms. Pain 1996;68:375–383.
22. Hedenberg-Magnusson B, Ernberg M, Kopp S. Symptoms and signs of temporomandibular disorders in patients with fibromyalgia and local myalgia of the temporomandibular system. A comparative study. Acta Odontol Scand 1997;55:344–349.