

# Plasma and Serum Serotonin Levels and Their Relationship to Orofacial Pain and Anxiety in Fibromyalgia

## Malin Ernberg, DDS

Graduate Student  
Department of Clinical Oral Physiology  
Karolinska Institutet  
Huddinge, Sweden

## Ülle Voog, DDS

Graduate Student  
Department of Clinical Oral Physiology  
Karolinska Institutet  
Huddinge, Sweden

Graduate Student  
Department of Stomatology  
University of Tartu  
Tartu, Estonia

## Per Alstergren, DDS, PhD

Assistant Professor  
Department of Clinical Oral Physiology  
Karolinska Institutet  
Huddinge, Sweden

## Thomas Lundeberg, MD, PhD

Associate Professor  
Department of Physiology and  
Pharmacology  
Karolinska Institutet  
Stockholm, Sweden

## Sigvard Kopp, DDS, PhD

Professor  
Department of Clinical Oral Physiology  
Karolinska Institutet  
Huddinge, Sweden

## Correspondence to:

Dr Malin Ernberg  
Department of Clinical Oral Physiology  
Institute of Odontology  
Box 4064  
S-141 04 Huddinge, Sweden  
E-mail: malin.ernberg@ofa.ki.se

**Aims:** Serum serotonin levels (S-5-HT) have been reported to be reduced in patients with fibromyalgia and to show a negative correlation with pain. We hypothesized that one mechanism behind this could be that platelets are activated to release 5-HT into the plasma compartment (P-5-HT), which then binds to nociceptors. The aims of this study were therefore to investigate the relation between P-5-HT and S-5-HT and their relationship versus orofacial pain and anxiety in fibromyalgia. **Methods:** Twelve patients with fibromyalgia, 12 patients with rheumatoid arthritis, and 12 healthy individuals participated in the study. Pain measures used were pain intensity assessed with a visual analog scale, pain drawings, and influence of pain on daily living activities (ADL). The Spielberger State and Trait Anxiety Inventory (STAI) score was used for self-rating of anxiety levels. The participants were examined clinically, and the pressure pain threshold (PPT) over the masseter muscle was assessed. Finally, venous blood was collected for analysis of P-5-HT and S-5-HT. **Results:** The ratio between P-5-HT and S-5-HT was calculated to determine the relative plasma fraction of serotonin (RPS). Patients with fibromyalgia showed significantly lower S-5-HT than did patients with rheumatoid arthritis. They also showed significantly higher STAI scores and tender point index of orofacial muscles and significantly lower PPT than the healthy individuals. High RPS was associated with high ADL and STAI scores. **Conclusion:** This study indicates that a high level of plasma serotonin in relation to serum level is associated with pain discomfort and increased anxiety in fibromyalgia.

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**Key words:** anxiety, fibromyalgia, serotonin, visual analog pain scale, pain threshold

Fibromyalgia is a generalized musculoskeletal pain condition characterized by muscle pain, tenderness, stiffness, and pronounced fatigue.<sup>1</sup> Other frequent symptoms are sleep disturbances, depression, and headache.<sup>2</sup> For research purposes, the diagnosis is usually established by the criteria of the American College of Rheumatology (ACR).<sup>2</sup> These criteria include pain for more than 3 months from all 4 body quadrants, axial skeletal pain, and pain upon digital palpation of at least 11 of 18 specific bilateral points. Several studies have shown that patients with fibromyalgia frequently suffer from orofacial pain and tenderness,<sup>3,4</sup> although none of the diagnostic tender points are located in this region.

Several studies have suggested that alterations in serotonergic neuronal function in the central nervous system (CNS) occur in patients with fibromyalgia, ie, that they suffer from a central

**Table 1** Distribution of Age and Duration of Local and General Symptoms (Mean  $\pm$  SEM) in 12 Female Patients with Fibromyalgia, 12 Female Patients with Rheumatoid Arthritis, and 12 Healthy Females

|                                  | Fibromyalgia | Rheumatoid arthritis | Healthy individuals |
|----------------------------------|--------------|----------------------|---------------------|
| Age (y)                          | 47 $\pm$ 2   | 47 $\pm$ 4           | 47 $\pm$ 2          |
| Duration of local symptoms (y)   | 16 $\pm$ 3   | 3 $\pm$ 1            | n.a.                |
| Duration of general symptoms (y) | 14 $\pm$ 2   | 10 $\pm$ 2           | n.a.                |

SEM = standard error of the mean; n.a. = not applicable.

deficiency of serotonin (5-HT). This is based on findings of an inverse association between plasma tryptophan (a precursor to 5-HT) and pain intensity,<sup>5</sup> a reduced level of plasma tryptophan, and a decreased transport ratio of tryptophan across the blood-brain barrier,<sup>6</sup> as well as a decreased cerebrospinal fluid (CSF) level of 5-hydroxyindoleacetic acid (5-HIAA), the metabolite of 5-HT.<sup>7</sup> Furthermore, the serum level of 5-HT (S-5-HT) has been reported to be lower in patients with fibromyalgia than in healthy controls.<sup>1,8,9</sup> However, in a recent study no significant difference in S-5-HT was found between patients with fibromyalgia or local myalgia, and age-matched healthy subjects,<sup>10</sup> in agreement with Moldofsky and Warsh,<sup>5</sup> who could not find a significant difference in plasma tryptophan between fibromyalgia patients and healthy individuals.

Apart from its association with pain, central depletion of 5-HT has also been reported to be associated with disturbed non-REM sleep and depression.<sup>11,12</sup> Krag et al<sup>13</sup> found that patients with fibromyalgia were more depressed than patients with low back pain and rheumatoid arthritis, but found no difference in plasma levels of serotonin (P-5-HT). Therefore they raised the question whether the lowered blood 5-HT found in previous studies was primary or secondary to pain and suggested that the psychiatric status in fibromyalgia should be interpreted cautiously.

Serotonin is synthesized from the essential amino acid tryptophan in the CNS and in the enterochromaffin cells of the small intestine. From the latter it is absorbed into the bloodstream and stored in platelets. A very small fraction, however, is freely circulating and biologically active in the blood plasma (P-5-HT). Serotonin is released from the platelets as a result of tissue trauma, for example, and plays a role in blood clotting and in the early phases of inflammation. Plasma levels of

5-HT of healthy individuals has been reported to vary between 0 and 11 nmol/L,<sup>14</sup> whereas S-5-HT, which consists both of 5-HT released from platelets during coagulation and the free fraction, is at least 100-fold higher. Platelets in the synovial fluid of patients with rheumatoid arthritis have been found to be activated, ie, to release 5-HT.<sup>15</sup> Furthermore, a high level of 5-HT in the synovial fluid of patients with rheumatoid arthritis has been found to be associated with pain provoked by joint movement.<sup>16</sup> Several 5-HT receptors have been identified, and some of them have been suggested to be involved in the mediation of pain and allodynia/hyperalgesia. It has been reported that pain from inflamed peripheral tissue is mediated by the 5-HT<sub>3</sub> receptor,<sup>17,18</sup> while allodynia/hyperalgesia is mediated by the 5-HT<sub>1A</sub> receptor.<sup>19</sup>

In a previous study we found a higher intramuscular level of 5-HT in the painful superficial masseter muscle of patients with fibromyalgia than in healthy individuals. We also found that a high level of 5-HT was associated with local pain and allodynia/hyperalgesia.<sup>20</sup> We hypothesized that a mechanism behind this could be that platelets are activated to release 5-HT into plasma, which then binds to nociceptors. The first aim of this study was therefore to investigate P-5-HT in relation to S-5-HT in patients with fibromyalgia. A second aim was to investigate their relationship to orofacial pain parameters and anxiety.

## Materials and Methods

### Subjects

Twelve female patients with fibromyalgia participated in the study (Table 1). All patients had participated in a special program at the Department of Rehabilitation Medicine at Karolinska Hospital,



Stockholm, where the diagnosis of fibromyalgia was determined according to the ACR criteria.<sup>2</sup> They were then referred to the Department of Clinical Oral Physiology for voluntary participation in this study. The patients were first given a brief clinical examination to evaluate whether they fulfilled the inclusion criteria. These were pain from the orofacial region for at least 3 months and tenderness of the orofacial muscles to digital palpation. Exclusion criteria were general inflammatory connective tissue disease (eg, rheumatoid arthritis) or symptoms that could be referred to disease in other parts of the orofacial area (eg, temporomandibular joints [TMJ], toothache, neuralgia). Three of the patients took antidepressant drugs (2 citalopram and 1 sertraline); 2 took ataractics (buspirone); 9 took analgesics (1 tramadol, 7 paracetamol, and 1 salicylic acid); and 2 took muscle relaxants (clorzoxazon).

Twelve healthy female individuals who were age-matched to the patients with fibromyalgia served as controls. They had no pain from the temporomandibular area, but minor tenderness to digital palpation was accepted as normal. None received medication.

Twelve age-matched female patients with rheumatoid arthritis, consecutively referred to the Department of Stomatology, Tartu University, Estonia, were included for comparison of P-5-HT and S-5-HT. They were included if they presented pain from any part of the TMJs for more than 6 weeks and presented tenderness of the TMJs to digital palpation. The diagnosis of rheumatoid arthritis was established according to the 1987 classification criteria of the American Rheumatism Association.<sup>21</sup> Three patients took immunosuppressive medicine (1 methothrexate and 2 chlorokine phosphate) and 2 patients took analgesics (nonsteroidal anti-inflammatory drugs).

The methods and selection of patients were approved by the local ethical committee at Huddinge Hospital, Karolinska Institutet, Stockholm (151/93) and Tartu University (55/5, 12.02.1998). All individuals gave their verbal consent prior to examination.

## Procedures

**Assessment of Pain.** A 100-mm visual analog scale with endpoints marked by "no pain" and "worst pain ever experienced" was used by the patients with fibromyalgia to assess their current pain intensity from the masseter muscle region (right and left side separately) shortly before examination.<sup>22</sup> They were also asked to assess their current

pain intensity every morning and evening during 1 week after the examination in the same way. The sum of the pain intensity of the right and left sides was used in the analyses.

Pain drawings were used at examination to record the current pain distribution in the fibromyalgia patients.<sup>23</sup> The patients were asked to mark their pain distribution as precisely as possible for the body in general and for the head.

The influence of pain on activities of daily living (ADL) was assessed by the fibromyalgia patients with a modified ADL score<sup>24</sup>; this was done separately for the masseter muscle region and for the body in general. This score consists of 12 questions, each of which aims to evaluate the influence of pain on a particular daily activity, and ranges from 0 (activity without any pain discomfort) to 10 (activity impossible due to pain discomfort). Three of the questions (9 to 11) concern activities in which the masticatory system is involved (ie, chewing, swallowing, yawning), while the last question provides an estimate of how much the pain affects daily activities overall. The sum of the scores of the questions (maximum 110) was calculated for each patient (body in general and masseter muscle region separately) and is referred to as ADL<sub>sum</sub>.

**Anxiety Level.** The subjects used a Swedish version of the Spielberger State and Trait Anxiety Inventory Scale (STAI),<sup>25</sup> which has been reported to be reliable and valid, for rating of the anxiety level.<sup>26</sup> This scale consists of 2 subscales, each with 20 statements that measure state (A-state) and trait (A-trait) anxiety. State anxiety can be defined as a transitory emotional condition, ie, feelings of tension and apprehension associated with increased autonomic nervous system activity at a given time. Trait anxiety describes how a person generally feels, ie, the tendency to perceive stressful situations as threatening. The scale ranges between 1 and 4 units, and the sum of the score of the 20 questions is calculated for each patient, giving a total sum of 20 to 80 units. A higher score indicates a higher level of anxiety.

**Clinical Examination.** The degree of tenderness to digital palpation over the superficial masseter, anterior temporalis, lateral and medial pterygoid, and trapezius muscles, as well as over the insertion of the temporal tendon, was assessed. A 4-graded scale, modified after Russell et al,<sup>12</sup> was used where 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 the tenderness score (maximum 36) was calculated for each patient and referred to as the tender point index (TPI).<sup>12</sup>



**Table 2** Subjective and Clinical Characteristics of 12 Female Patients with Fibromyalgia and 12 Age-Matched Healthy Females

|   | Fibromyalgia | Healthy individuals |
|---|--------------|---------------------|
| Pain intensity, 1 week (0 to 100) (median and IQR)      | 74 (99)      | n.a.                |
| Pain intensity, examination (0 to 100) (median and IQR) | 53 (76)      | n.a.                |
| TPI (0 to 36) (median and IQR)                          | 19 (3)       | 1 (2)               |
| PPT <sub>sum</sub> (kPa) (mean and SEM)                 | 216 (26)     | 426 (29)            |

Pain intensity = sum of the pain from the right and left masseter muscle region assessed with a visual analog scale twice every day during 1 week and at examination; TPI = sum of palpation scores regarding masticatory muscles; PPT<sub>sum</sub> = sum of the pressure pain threshold of the right and left masseter muscles; IQR = interquartile range; SEM = standard error of the mean; n.a. = not applicable. Significant difference between groups regarding TPI and PPT<sub>sum</sub> ( $P < 0.001$ ).

The pressure pain threshold (PPT) was assessed (kPa) with an electronic algometer (Somedic Sales AB) over the most tender point of the superficial masseter muscles. The algometer had a blunt rubber recording tip of 1 cm<sup>2</sup>. The algometer was held perpendicular to the skin surface, and the pressure was increased slowly at a pressure rate of 50 kPa/s. Subjects were instructed to press a button as soon as the sensation of pressure changed to pain. This was first done over the soft tissue close to the base of the thumb on the dorsal side of the right hand to accustom the subject to the procedure. Three recordings of PPT were then made, 2 minutes apart, over the 2 superficial masseter muscles. The mean of the 3 recordings was used in the analyses. The PPT was defined as the pressure applied when the patient reported that the sensation of pressure changed to pain. The sum of the PPT values from the right and left was calculated for each patient and referred to as PPT<sub>sum</sub>.

**Blood Examination.** Venous blood was collected in an EDTA-containing tube for the platelet count and in a tube without additives for C-reactive protein (S-CRP). These samples were analyzed at the Department of Clinical Chemistry, Huddinge Hospital. None of the patients with fibromyalgia or the healthy individuals showed elevated levels of CRP, while 3 of the patients with rheumatoid arthritis did. A sample of 2 mL was collected in a tube without additives for analysis of S-5-HT. This blood sample was left at room temperature for 1 hour to coagulate and then cold-centrifuged (+4°C, 1,700g) for 30 minutes. Another sample of 5 mL was collected for analysis of the platelet-poor plasma level of 5-HT in an EDTA-containing tube,

which was immediately cooled and centrifuged (+4°C, 1,700g) for 30 minutes. Approximately 200  $\mu$ L of the supernatant from the 2 tubes was then pipetted into polystyrene tubes and kept frozen at -22°C until analysis. To avoid interference with the analysis of 5-HT, the patients were asked to avoid tryptophan-rich food (eg, banana, pineapple, tomato, and chocolate) for 24 hours before the examination. Due to the diurnal variation of 5-HT,<sup>27</sup> all blood samples were collected in the afternoon.

To determine the relationship between P-5-HT and S-5-HT, the ratio between these 2 variables was calculated according to the following formula and referred to as the relative plasma fraction of serotonin (RPS):

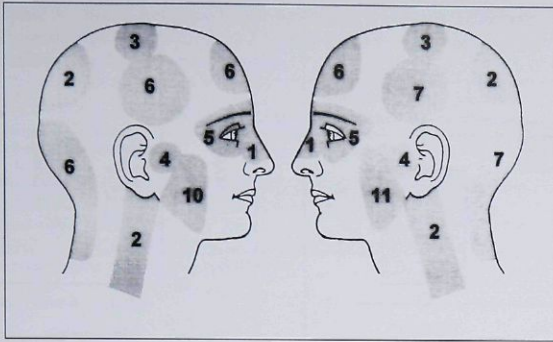
$$RPS = \frac{P-5-HT \times 100}{S-5-HT}$$

This expresses the percentage of free-circulating 5-HT in the blood in relation to the total 5-HT content.

**Biochemical Analysis.** Serotonin levels were analyzed at the Department of Clinical Oral Physiology, Huddinge, by a commercially available competitive enzyme immunoassay kit (No. 0642, Immunotech International). The kit has a sensitivity of 0.5 nmol/L and is applicable at concentrations between 1.6 and 200 nmol/L. According to the manufacturer, the intra-assay coefficient of variation varies between 7.9 and 9.4% and the inter-assay coefficient of variation varies between 6.2 and 9.9%. The S-5-HT samples were diluted 1:20 prior to analysis and the values obtained were corrected for the dilution.

## Statistics

The Kolmogorov-Smirnov test for continuous variables was used to test the normality of the variables. One-way analysis of variance with the Tukey test for pairwise multiple comparisons or Student's unpaired *t* test was used to test the significance of the difference between groups regarding 5-HT levels, RPS, and platelet count. The Wilcoxon test was used to test the differences between ADL for the body in general and orofacial region in the patients with fibromyalgia. Student's unpaired *t* test was used to test the difference in PPT, and the Mann-Whitney *U* test was used to test the difference in STAI scores and TPI between patients with fibromyalgia and healthy individuals. The Pearson correlation test was used to test the relationship



**Fig 1** The distribution of current head and neck pain reported by 11 female patients with fibromyalgia. The gray areas represent anatomic locations where pain was present, and the numerals indicate the number of patients reporting pain from that area. Two patients reported pain from the forehead, temples, and occipital regions, ie, "as a taut band around the head."

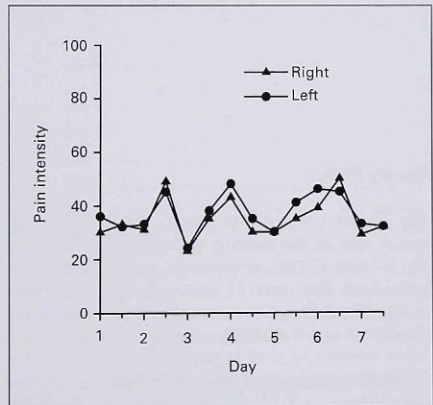
between 5-HT levels, RPS, STAI, and normally distributed data and data measured on an interval scale (age, duration of pain, PPT, and platelet count). Spearman's rank correlation test was used to test the relationship between 5-HT levels, RPS, STAI and non-normally distributed data, and data measured on an ordinal scale (pain intensity, ADL, STAI, and TPI). All tests were 2-tailed, and the significance level was set at  $P < 0.05$ .

## Results

### Pain and Allodynia/Hyperalgesia

The results from the clinical examination are shown in Table 2. All fibromyalgia patients reported pain from all 4 body quadrants and extremities on the pain drawings. All but 1 patient reported bilateral pain from the head (Fig 1). The regions of the head most frequently reported painful were the cheek, neck, temple, forehead, and TMJ. Two patients reported pain with a similar distribution as tension headache, ie, "as a taut band around the head." The pain intensity over the masseter muscle region assessed during 1 week varied between recordings (Fig 2).

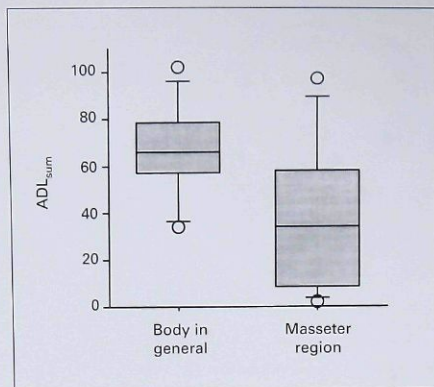
The fibromyalgia patients had significantly higher ADL scores for the body in general than for the masseter muscle region on questions 1 to 8 and 12 ( $0.003 \leq P \leq 0.018$ ), while there were no signif-



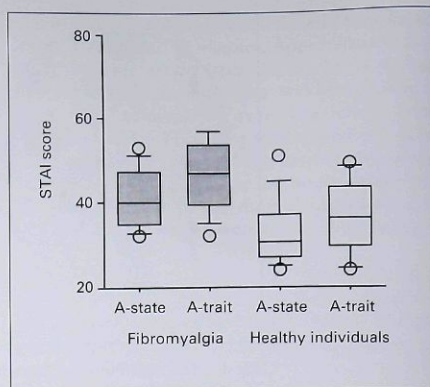
**Fig 2** Median pain intensity assessed with a visual analog scale (0 to 100) by 11 female patients with fibromyalgia. Pain was assessed in the right and left masseter muscle region every morning and evening during 1 week, starting on the morning of Day 1.

icant differences regarding questions 9 to 11. Also, ADL<sub>sum</sub> was significantly higher for the body in general than for the masseter muscle region ( $P = 0.004$ ; Fig 3).





**Fig 3** Box plots (10th, 25th, 50th, 75th, and 90th percentile) showing the influence of pain on daily activities assessed separately for the body in general and for the masseter muscle region by 11 female patients with fibromyalgia. ADL<sub>sum</sub> expresses the sum of the score of the first 11 questions on the questionnaire (0 to 110). There was a significant difference between ADL<sub>sum</sub> for the body in general and for the masseter muscle region ( $P = 0.004$ ).



**Fig 4** Box plots (10th, 25th, 50th, 75th, and 90th percentile) showing the level of anxiety reported by 11 female patients with fibromyalgia and 12 age-matched healthy female individuals. Anxiety was assessed with the Spielberger State and Trait Anxiety Inventory Scale (STAI), ranging between 20 and 80. A-state measures a transitory state of anxiety, while A-trait measures the tendency to perceive stressful situations as threatening. There were significant differences between the patients and healthy individuals as regards both A-state and A-trait ( $P = 0.014$  and  $P = 0.020$ , respectively).

### Anxiety Status

The median (interquartile range) values for A-state and A-trait in the patients with fibromyalgia were 40 (14) and 47 (16), respectively, and in the healthy individuals they were 31 (11) and 36 (17), respectively. The differences were statistically significant ( $P = 0.014$  and  $P = 0.020$ , respectively; Fig 4).

### Correlation Between Anxiety and Pain Parameters

There were positive correlations between STAI and pain parameters, with statistical significance between A-state and the pain intensity scored during 1 week ( $r_s = 0.69$ ,  $n = 11$ ,  $P = 0.018$ ), as well as TPI ( $r_s = 0.63$ ,  $n = 11$ ,  $P = 0.039$ ) in the patients with fibromyalgia.

### Levels of Serotonin

Values for S-5-HT, P-5-HT, and RPS are shown in Table 3. The 3 groups differed as regards S-5-HT ( $P = 0.013$ ), with a significant difference between

the patients with fibromyalgia and rheumatoid arthritis ( $P < 0.05$ ). There were no significant differences between the groups in P-5-HT or RPS. The S-5-HT and P-5-HT of the fibromyalgia patients taking antidepressant or ataractic medication were lower (30% and 62%, respectively) than in those who did not take these drugs, but the difference was not statistically significant. Platelet counts were within the normal range and did not differ between groups. There was no significant correlation between the number of platelets and S-5-HT levels.

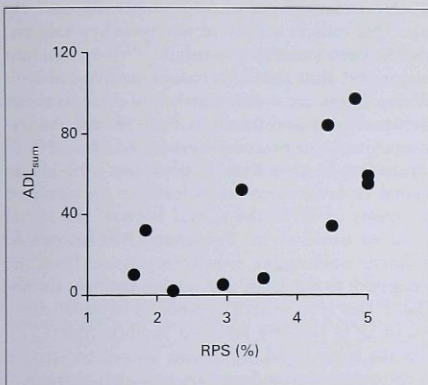
### Correlation Between Serotonin, Pain, and Anxiety

In the patients with fibromyalgia, there were positive correlations between RPS and ADL<sub>sum</sub> for the body in general ( $r_s = 0.67$ ,  $n = 11$ ,  $P = 0.022$ ) and masseter muscle region ( $r_s = 0.71$ ,  $n = 11$ ,  $P = 0.015$ ; Fig 5) as well as between RPS and A-trait ( $r_s = 0.68$ ,  $n = 11$ ,  $P = 0.020$ ; Fig 6). Plasma serotonin level was negatively correlated to PPT<sub>sum</sub> ( $r_s = -0.61$ ,  $n = 12$ ,  $P = 0.032$ ) in the healthy individuals.

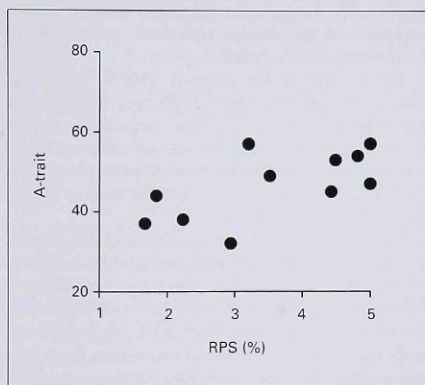
**Table 3** Blood Levels of Serotonin (Mean  $\pm$  SEM) in 12 Female Patients with Fibromyalgia, 12 Female Patients with Rheumatoid Arthritis, and 12 Healthy Females

|                                 | Fibromyalgia   | Rheumatoid arthritis | Healthy individuals |
|---------------------------------|----------------|----------------------|---------------------|
| Serum serotonin level (nmol/L)  | 581 $\pm$ 121  | 1282 $\pm$ 214       | 789 $\pm$ 132       |
| Plasma serotonin level (nmol/L) | 20.2 $\pm$ 4.7 | 36.1 $\pm$ 5.9       | 30.0 $\pm$ 6.3      |
| Relative plasma fraction (%)    | 3.9 $\pm$ 0.5  | 3.7 $\pm$ 0.9        | 4.4 $\pm$ 0.9       |

SEM = standard error of the mean. Significant difference between fibromyalgia and rheumatoid arthritis regarding serum serotonin level ( $P < 0.05$ ).



**Fig 5** Scatter plot showing the association between the relative plasma fraction of serotonin (RPS) and ADL<sub>sum</sub> for the masseter muscle region in 11 female patients with fibromyalgia. RPS = the ratio between plasma and serum level of 5-HT and expresses the percentage of free circulating 5-HT in the blood in relation to the total 5-HT content; ADL<sub>sum</sub> expresses the influence of pain on activities of daily living and is calculated as the sum of the score of the first 11 questions on the questionnaire (0 to 110). The correlation was statistically significant ( $r_s = 0.71$ ,  $n = 11$ ,  $P = 0.015$ ).



**Fig 6** Scatter plot showing the association between the relative plasma fraction of serotonin (RPS) and anxiety level in 11 female patients with fibromyalgia. RPS = the ratio between plasma and serum level of 5-HT, which expresses the percentage of free circulating 5-HT in the blood in relation to the total 5-HT content. The anxiety level was assessed with the Spielberger State and Trait Anxiety Inventory Scale, ranging between 20 and 80. A-trait expresses the tendency to perceive stressful situations as threatening. The correlation was statistically significant ( $r_s = 0.68$ ,  $n = 11$ ,  $P = 0.020$ ).

## Discussion

In this study, patients with fibromyalgia showed significantly lower S-5-HT than patients with rheumatoid arthritis. This is in agreement with a previous study,<sup>9</sup> and with the report of 2-fold lower levels of 5-HIAA in the CSF of patients with fibromyalgia than in patients with rheumatoid arthritis.<sup>7</sup> To our knowledge, there is only 1 previous study of P-5-HT in fibromyalgia, which

reported similar levels in these patients compared to rheumatoid arthritis.<sup>13</sup> There was no significant difference in S-5-HT or P-5-HT between patients with fibromyalgia and healthy individuals in our study, which is in agreement with the study above and our previous result from another patient sample.<sup>10</sup> However, there are several reports of lower blood tryptophan and 5-HT levels in fibromyalgia.<sup>6-9</sup> The divergent results are difficult to explain. In this study the groups were carefully controlled



for gender,<sup>28</sup> age,<sup>28</sup> and diurnal variation of 5-HT,<sup>27</sup> as well as for food intake of tryptophan.<sup>29</sup> It has recently been reported that the 5-HT reuptake inhibitor (SSRI) fluoxetine significantly decreased blood 5-HT levels in depressed patients.<sup>30</sup> Since some fibromyalgia patients were on medication with SSRI and buspirone (a 5-HT<sub>1A</sub> receptor agonist) we also tested the difference in 5-HT levels between these patients and the other fibromyalgia patients. Our study also showed lower 5-HT and P-5-HT in the patients under medication, but the difference was not significant. We also tested whether exclusion of these patients would alter the results, but the results remained similar. We therefore consider this factor of minor influence in this study, and these patients were accordingly not excluded.

The P-5-HT in the patients with fibromyalgia and in the healthy individuals was higher than reported previously.<sup>14,31</sup> The reason for this is unknown, but it could be due to different techniques used for preparing platelet-poor plasma, ie, centrifugation time and g values as well as different methods for analysis.

The RPS was similar in patients with fibromyalgia and rheumatoid arthritis and healthy individuals, which is in agreement with a previous study.<sup>13</sup> However, high RPS in the patients with fibromyalgia was associated with high ADL, ie, behavioral response to pain. This could mean that there is a subgroup of patients with fibromyalgia in which platelets are activated to release 5-HT or which has more receptors expressed. The 5-HT may then bind to peripheral 5-HT<sub>3</sub> receptors, which are known to elicit pain in humans.<sup>18</sup> This is in agreement with a recent study of systemic treatment with the 5-HT<sub>3</sub> receptor antagonist ondansetron, where a positive effect was found on pain and allodynia/hyperalgesia in about half the patients.<sup>18</sup> Therefore 2 subsets of patients with regard to disturbance of the 5-HT<sub>3</sub> receptor system may exist, ie, 1 group where the 5-HT<sub>3</sub> receptors are up-regulated, and another group where other mediators are more important.<sup>18,32</sup>

The anxiety level in the healthy individuals was similar to that reported before<sup>26</sup> and significantly lower than in the patients with fibromyalgia. Depressive symptoms have been found to be more common in fibromyalgia than in healthy individuals in several studies.<sup>33-35</sup> Our result in this aspect thus supports previous findings. Furthermore, a high level of anxiety was correlated to high pain intensity, high TPI, and high RPS. There are many reports of an association between low 5-HT level and depression,<sup>36,37</sup> as well as fibromyalgia,<sup>1,9</sup> and

that chronic pain is associated with anxiety and depressive symptoms.<sup>13,38,39</sup> It has also been reported that a high level of anxiety reduced pain relief after transcutaneous electrical nerve stimulation.<sup>40</sup> One explanation to this complex association between pain, anxiety, and 5-HT level could be an interaction between 5-HT and the neuropeptide cholecystokinin, which acts as an endogenous opioid antagonist. It has been found that high anxiety is associated with increased release of cholecystokinin and that this release is blocked by the 5-HT<sub>3</sub> receptor antagonist ondansetron.<sup>41</sup>

The patients with fibromyalgia had a lower PPT of the masseter muscle and a higher number of tender orofacial muscles than the healthy individuals. This reflects a state of allodynia/hyperalgesia, as has been stated previously.<sup>4,10,42,43</sup> It has been suggested that the lowered pain thresholds in fibromyalgia are a consequence of an interaction between 5-HT and substance P (SP) in the endogenous pain-modulating system of the CNS.<sup>32</sup> According to that theory, depletion of 5-HT at spinal or brain stem levels leads to an increased synthesis of SP in the dorsal horn of the spinal cord or brain stem. Substance P is known to enhance nociceptive pain transmission from the periphery to the CNS and lowers the pain threshold.<sup>37</sup> This is supported by findings of elevated levels of SP in the CSF reported in fibromyalgia.<sup>44,45</sup> On the other hand, SP is also known to activate serotonergic descending pain-modulating tracts.<sup>46</sup> Thus, high levels of SP in combination with low levels of 5-HT might reflect a failure to inhibit pain transmission. Remarkably, high P-5-HT was associated with low PPT in the healthy individuals. This indicates that the pain threshold of non-painful muscles is determined to a certain extent by the free amount of 5-HT available in blood.

All fibromyalgia patients reported pain from all 4 body quadrants, including all extremities, and 11 of the 12 had bilateral pain from the orofacial region. The masseter region was the most commonly painful orofacial region, which could be expected, since pain from this region belonged to the inclusion criteria. Apart from this region, pain was reported to be widespread over the head and neck. Even though none of the diagnostic tender points in fibromyalgia are located in the orofacial region, previous studies have already shown that pain from this area is common.<sup>3,4</sup> The pain assessed shortly before examination was lower than the median pain assessed over the following week, although the pain intensity from the masseter muscle region varied over the day and week for all patients. The latter condition could be



expected due to the fluctuating nature of chronic pain. Interestingly, though, the variation was very symmetric, ie, the pain intensity on both sides was similar. The patients scored the influence of pain on daily living activities higher for the body in general than for the masseter muscle region, except for activities specific to the masticatory system. This is natural, since most important activities in daily work and household chores involve other muscles of the body than the masticatory muscles, which are involved mainly with chewing, swallowing, and yawning. In a previous study we also found that the pain intensity reported by patients with fibromyalgia was judged to be higher in other parts of the body than in the masseter muscle region.<sup>10</sup>

This study indicates that the relationship between plasma and serum levels of 5-HT is similar in patients with fibromyalgia and healthy individuals, but that a high level of plasma serotonin in relation to serum level is associated with pain, discomfort, and increased anxiety in fibromyalgia.

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