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Catecholamine and Indolamine Pathway: A Case–Control Study in Fibromyalgia

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Abstract

Objectives: Fibromyalgia (FM) is a complex syndrome characterized by widespread pain. Its etiology is unclear, and diagnosis is difficult. The aim of this study was to assess plasma levels of monoamine neurotransmitters (catecholamines, indolamines, and intermediate metabolites) in patients with FM and healthy controls to investigate possible alterations in the metabolism of these molecules in FM. We also examined potential relationships between monoamine neurotransmitters and clinical features of FM. The predictive value of these molecules in FM was determined by receiver operating characteristic analysis. **Method:** We measured plasma catecholamines (epinephrine, norepinephrine, and dopamine), as well as indolamines and intermediary metabolites (serotonin or 5-hydroxytryptamine [5-HT], 5-hydroxyindolacetic acid [5-HIAA], 5-hydroxytryptophan [5-HTP], and *N*-acetyl-5-hydroxytryptamine [Nac-5-HT]) in 35 women with FM and 12 age-matched healthy women. **Results:** Higher levels of norepinephrine and lower levels of dopamine, 5-HT, 5-HIAA, and 5-HTP were found in women with FM in comparison with controls. Epinephrine and Nac-5-HT levels did not differ significantly between groups. Higher norepinephrine levels were associated with worse physical health status in FM patients. Also, plasma norepinephrine levels > 694.69 pg/ml might be an accurate predictor of FM. **Conclusions:** These findings show evidence of the dysregulation of the catecholamine and indolamine pathway in patients with FM, which may contribute to the physiopathology of this syndrome. In addition, the determination of plasma norepinephrine levels could help in the FM diagnosis.

Keywords

epinephrine, norepinephrine, dopamine, serotonin, fibromyalgia

Fibromyalgia (FM), which disproportionately affects women, is characterized by widespread chronic musculoskeletal pain. It is often associated with muscle stiffness, persistent fatigue, sleep disturbance, reduced physical strength, and psychiatric disorders such as depression. Its prevalence within the general population is 1.3–8%, making it the second most common rheumatologic disorder behind osteoarthritis (reviewed in Chinn, Caldwell, & Gritsenko, 2016). Patients with FM report severe disability and are high utilizers of health-care resources (Penrod et al., 2004).

Although the pathogenesis of FM remains unknown, researchers have proposed a number of factors that might contribute to its development. These factors include abnormal regulation of the central pain modulation system and altered levels of neurotransmitters (reviewed in Clauw, Arnold, & McCarberg, 2011), neuroendocrine disorders (Bote, García, Hinchado, & Ortega, 2012), and others that our research group previously investigated, including oxidative stress (La Rubia, Rus, Molina, & Del Moral, 2013) and inflammation (Rus et al., 2016).

Neurochemical imbalances due to altered levels of neurotransmitters in patients with FM have been associated with the dysregulation of pain perception characterized by allodynia and hyperalgesia (reviewed in Clauw et al., 2011). One such group of neurotransmitters is the monoamine neurotransmitters, which derive from aromatic amino acids and include catecholamines and indolamines. Catecholamines derive from phenylalanine and include dopamine, norepinephrine, and epinephrine, while the indolamines are synthesized from tryptophan and include serotonin or 5-hydroxytryptamine (5-HT). Catecholamines are produced by the adrenal medulla and the postganglionic fibers of the sympathetic nervous system (Petzke & Clauw, 2000). The sympathetic nervous system is implicated in the stress response of the body throughout the delivery of catecholamines. Several studies have suggested that patients with FM have an inadequate response to stressful

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situations when compared to healthy subjects due to the dysregulation of the sympathetic nervous system, though findings are conflicting, ranging from sympathetic hypoactivation to overactivation (Kadetoff & Kosek, 2010; Martinez-Lavin, 2007; Petzke & Clauw, 2000). In addition, specific FM symptoms have been related to sympathetic dysfunction (Zamunér et al., 2015). For example, 5-HT is synthesized in the gastrointestinal tract and in serotonergic neurons of the central nervous system, where it regulates mood, appetite, sleep, and cognitive functions such as memory and learning. 5-HT also plays a critical role in descending pain pathways related to pain inhibition (Millan, 2002) and has been related to pain in patients with FM (Ernberg, Voog, Alstergren, Lundeberg, & Kopp, 2000; Russell, Michalek, et al., 1992).

Previous research has shown conflicting results as to plasma levels of catecholamines and indolamines in patients with FM. Investigators have reported levels of dopamine, norepinephrine, and epinephrine to be increased, decreased, and unchanged in subjects with FM versus healthy controls (Bote et al., 2012; Giske et al., 2008; Kadetoff & Kosek, 2010; Light et al., 2009; Torpy et al., 2000). Similarly, some authors have reported reduced levels of 5-HT in FM compared with the control group (Bote et al., 2012), while others did not find differences between patients and healthy subjects (Paul-Savoie et al., 2011). In addition, the available studies have each determined the levels of only one or a few of these neurotransmitters in patients with FM. To the best of our knowledge, no study has measured the levels of all these molecules in the same cohort of FM patients. Therefore, our aim in the present study was to assess plasma levels of catecholamines, indolamines, and intermediate metabolites in patients with FM and healthy controls in order to investigate possible alterations in the metabolism of these molecules in FM. We also examined the possible relation of monoamine neurotransmitters with specific clinical features of FM to address whether levels of catecholamines and indolamines may influence clinical characteristics in patients with FM. We determined the predictive value of these molecules in FM by receiver operating characteristic (ROC) analysis.

Method

Participants

We conducted this research in accordance with the World Medical Association (WMA) Declaration of Helsinki (2008) of the World Medical Association. The Ethics Committee of the University of Jaén (Spain) approved the study, and all subjects provided written informed consent. We recruited 35 female patients with FM from Association of Fibromyalgia of Jaén (AFIXA; Spain) and 12 age-matched healthy women from the University of Jaén (Spain) to participate in the study.

The inclusion criteria for the FM group were that all of the patients meet the 1990 American College of Rheumatology (ACR) criteria for the classification of primary FM. Exclusion criteria for both groups included the presence of any other chronic disease (diabetes mellitus, hypertension, cancer, and ischemic heart disease), pregnancy, lactation, and Grade II obesity (body mass index [BMI] $\geq$ 35 kg/m^2). None of the participants were currently receiving treatment with corticosteroids, estrogens, analgesics, or anti-inflammatory drugs and had used them in the 2 months prior to the start of the study. None consumed alcohol regularly, and all were nonsmokers. All of the participants had sedentary lifestyles.

Clinical Characteristics of Participants

We obtained demographic and clinical data from patient interview and questionnaires. The same specialist carried out all the measurements and tests throughout the study. In patients with FM, we evaluated functional capacity in daily living activities using the Spanish version of the Fibromyalgia Impact Questionnaire (FIQ; Cronbach's α coefficient = .82; Rivera & González, 2004). Scores on the FIQ, which is used by the instrument researchers most often to estimate FM severity, range from 0 to 100. To measure musculoskeletal pain, we used a Visual Analogue Scale (VAS; 10 cm). For both of these instruments, higher scores reflect worse symptomatology. We assessed the physical (Physical Component Summary, PCS-12; Cronbach's α coefficient = .85) and mental (Mental Component Summary, MCS-12; Cronbach's α coefficient = .78) health status (Vilagut et al., 2008) of patients and controls using the Spanish version of the 12-Item Short-Form Health Survey (Alonso, Prieto, & Anto, 1995). Scores range from 0 to 100, with lower values reflecting worse health status.

Blood Collection and Preparation of Blood Samples

We drew venous blood from the antecubital vein into an ethylenediaminetetraacetic acid tube (BD Vacutainer® LH PST II Advance, Ref. 367374, Becton Dickinson, New Jersey, USA) in the early morning after an overnight fast, as recommended. We collected blood from all participants at the same time of the day to avoid the potentially confounding factor of daily variations in the levels of catecholamines and indolamines. We centrifuged the tube at 3,500 rpm for 5 min at 4°C to obtain plasma. Then, we collected the supernatants, aliquoted them, and kept them at −80°C until analyses.

Determination of Levels of Catecholamines and Indolamines

We determined the levels of the catecholamines (epinephrine, norepinephrine, and dopamine) simultaneously with those of the indolamines and intermediate metabolites (5-HT, 5-hydroxyindolacetic acid [5-HIAA], 5-hydroxytryptophan [5-HTP], and *N*-acetyl-5-hydroxytryptamine [Nac-5-HT]) using high-performance liquid chromatography with fluorometric precolumn derivatization, as previously described by Iizuka, Ishige, and Ohta Yajima (1999) with minor modifications. The investigator who performed these determinations was blinded to the identity of the participant who provided the samples. Briefly, plasma samples were deproteinated by

Table 1. Clinical Characteristics of Women With FM and Healthy Women.

Characteristic	Healthy Controls ($n = 12$)	Patients With FM ($n = 35$)	*P* Value	Effect Size	Power
Systolic BP (mmHg)	113.57 ± 10.29	119.38 ± 13.95	.307	0.3	0.151
Diastolic BP (mmHg)	67.86 ± 6.98	74.06 ± 10.65	.126	0.055	0.052
MCS-12	48.63 ± 6.72	39.21 ± 12.98	.025	0.1	0.437
PCS-12	52.78 ± 7.31	30.50 ± 7.74	<.001	>2.0	1.0
FIQ	—	60.73 ± 14.73	—	—	—
VAS	—	6.16 ± 2.21	—	—	—

Note. Data are expressed as mean ± standard deviation. BP = blood pressure; FIQ = Fibromyalgia Impact Questionnaire; FM = fibromyalgia; MCS-12 = Mental Health Component Summary of SF-12 Survey; PCS-12 = Physical Component Summary of SF-12 Survey; SF-12 Survey = 12-Item Short-Form Health Survey; VAS = Visual Analog Scale.

ultrafiltration through a 10,000 molecular weight cutoff filter. The deproteinated plasma underwent precolumn derivatization with a mixture of 4-dimethylaminobenzylamine 240 mM, $K_3[Fe(CN)_6]$ 5.3 mM, and NaOH 300 mM in 200 µl tert-butyl alcohol and was heated for 15 min at 60°C. Then, 20 µl of the solution was injected through a refrigerated PerkinElmer Series 200 automatic sample injector into a 150 × 3.9 mm Waters Resolve 5 µm C18 column. The mobile phase consisted of acetate buffer 10 mM (pH = 4.5) containing 1 mM octanesulfonic acid/acetonitrile (60:40) at a flow rate of 0.8 ml/min in isocratic mode using a PerkinElmer Series 200 pump for 30 min. The fluorescence detector (PerkinElmer Series 200a) was set at an excitation wavelength of 360 nm and an emission wavelength of 440 nm. Data were processed with the TotalChrom WorkStation Version 6.3.1 software from PerkinElmer.

Statistical Analysis

We have expressed data for continuous variables as mean ± standard deviation. Management and data analysis were performed using the statistical package IBM SPSS Statistics Version 24 for Windows (SPSS Inc., Chicago, IL). We performed the Kolmogorov–Smirnov test (α value = .05) and Levene test (α value = .05) to test normality and homoscedasticity, respectively. We tested data that followed a normal distribution and the principle of homoscedasticity of variances using an unpaired Student's *t* test to compare differences between means. To establish the degree of statistical significance in data that did not follow a normal distribution or the principle of homoscedasticity (5-HTP, Nac-5-HT, MCS-12, and diastolic blood pressure [BP]), we applied the Mann–Whitney *U* test. To calculate the effect size, we used Cohen's *d* for parametric tests and eta squared (η^2) for nonparametric tests. Values of Cohen's *d* of .2, .5, and .8 correspond to the classical Cohen bands of interpretation of the effect size as small, medium, and large, respectively. Sawilowsky (2009) recommended expanding Cohen's rules of thumb for interpreting effect sizes to include very small (0.01), very large (1.2), and huge (2.0) effect sizes. Values of η^2 of .02, .13, and .26 correspond to small, moderate, and large effect sizes, respectively. We performed power analyses for parametric and nonparametric tests using G*Power Version 3.1.9.2 software (a value greater than .8 is a high power). To assess the relationships between variables (biochemical markers and clinical parameters), we used Pearson's and Spearman's correlation coefficients as parametric and nonparametric measures of rank correlation, respectively. We set the level of statistical significance at $p < .05$. We used MedCalc statistical software to calculate ROC curves, cutoff point, positive and negative predictive values (PV+ and PV−, respectively), sensitivity, and specificity. The Youden index was used to determine the cutoff point of the variable in the ROC curve (Youden, 1950).

Results

Participants

Table 1 summarizes the clinical data of the participants. There were no statistically significant differences in age (50 ± 8.3 years for the controls and 52.3 ± 7.8 years for the FM patients) or BMI (24.07 ± 3.05 kg/m^2 for the controls and 26.14 ± 3.48 kg/m^2 for the FM patients) between the two groups. There were no significant differences in systolic or diastolic BP between patients with FM and healthy subjects either. Systolic and diastolic BP had small-to-medium effect sizes and low power. The PCS-12 and MCS-12 scores of women with FM were significantly lower compared with those of controls ($p < .001$ and $p = .025$, respectively). The PCS-12 had a huge effect size and high power, while the MCS-12 had a medium effect size and low power. For FM patients, the mean FIQ score was 60.73 ± 14.73, and the mean VAS score was 6.16 ± 2.21.

Determination of Levels of Catecholamines and Indolamines

Table 2 summarizes the results for plasma levels of catecholamines, indolamines, and intermediate metabolites. Results showed significantly higher plasma levels of norepinephrine in women with FM in comparison with controls ($p = .003$) and lower levels of dopamine, 5-HT, 5-HTP, and 5-HIAA (all $p < .001$) in patients versus healthy women. All these variables showed effect sizes ranging from large to huge. There were no significant differences in levels of epinephrine and Nac-5-HT between the groups, and these variables had small effect sizes and low power.

Table 2. Plasma Levels of Catecholamines, Indolamines, and Intermediate Metabolites in Women With FM and Healthy Women.

Measure	Healthy Controls ($n = 12$)	Patients with FM ($n = 35$)	*P* Value	Effect Size	Power
Epinephrine (pg/ml)	47.40 ± 17.78	41.27 ± 16.35	.301	0.253	.222
Norepinephrine (pg/ml)	657.75 ± 149.35	791.19 ± 107.79	.003	0.849	.991
Dopamine (pg/ml)	83.42 ± 17.72	48.71 ± 19.20	<.001	1.853	.998
5-HT (ng/ml)	116.30 ± 49.30	63.70 ± 23.04	<.001	1.274	.989
5-HTP (ng/ml)	10.72 ± 4.21	4.74 ± 2.30	<.001	0.3	.955
5-HIAA (ng/ml)	6.20 ± 2.12	3.25 ± 1.36	<.001	1.544	.996
Nac-5-HT (ng/ml)	9.01 ± 2.53	11.91 ± 5.20	.198	0.02	.123

Note. Data are expressed as mean ± standard deviation (*SD*). FM = fibromyalgia; 5-HIAA = 5-hydroxyindolacetic acid; 5-HT = serotonin; 5-HTP = 5-hydroxytryptophan; Nac-5-HT = *N*-acetyl-5-hydroxytryptamine.

Table 3. Correlations Between Norepinephrine Levels and Clinical Features in Women With Fibromyalgia.

Clinical Feature	Norepinephrine
PCS-12	$r = -.368$, $p = .038$
Systolic BP	$r = .467$, $p = .007$
Diastolic BP	$\rho = .339$, $p = .058$

Note. $n = 35$. BP = blood pressure; PCS-12 = Physical Component Summary of the Short-Form (SF)-12 Health Survey; r = Pearson's correlation coefficient; ρ = Spearman's correlation coefficient.

Correlations Between Levels of Catecholamines and Indolamines and Clinical Features

Plasma levels of norepinephrine had a significant negative correlation with score on the PCS-12 and positive correlation with systolic BP in women with FM (Table 3). The positive correlation between norepinephrine levels and diastolic BP approached statistical significance in FM patients. There were no significant correlations between biochemical markers and health-related parameters in the healthy women.

ROC Curves

Figure 1 shows the ROC curves for plasma levels of catecholamines and indolamines. The area under the curve (AUC) was greater than .75 only for norepinephrine levels (AUC = .819, 95% confidence interval [.660, .978]), which means that, of these measures, only norepinephrine would be a good marker for differentiating individuals with FM from healthy subjects. That is, there is more than an 80% probability that, given two randomly chosen individuals, one with FM and one a healthy individual without FM, norepinephrine level would distinguish between the two. The cutoff point was 694.69 pg/ml, the PV+ was 89.7, and the PV− was 53.8. The predictive values indicate that almost 90% of subjects who have norepinephrine levels above 694.69 pg/ml may be diagnosed with FM with high sensitivity and specificity and that the probability of cataloguing a person with levels of norepinephrine below 694.69 pg/ml as healthy is more than 50%.

Discussion

FM is very difficult to diagnose and treat due to its unclear physiopathology. Therefore, a better understanding of the underlying mechanisms of FM is necessary to more effectively diagnose and manage this syndrome. In the present study, we examined plasma levels of catecholamines and indolamines in patients with FM and healthy controls to investigate possible alterations in their metabolism that may be related to FM.

We found that women with FM presented significantly higher plasma levels of norepinephrine and lower plasma levels of dopamine than those of controls. Epinephrine levels were similar between patients and healthy subjects. These findings suggest that the catecholamine pathway is altered in patients with FM, which may be related to the dysregulation of the sympathetic nervous system. Previous researchers have reported an association between FM and catecholamine metabolism, but results have been inconsistent across studies, with findings of increased, decreased, and unchanged plasma levels of dopamine, norepinephrine, and epinephrine in subjects with FM versus healthy controls (Bote et al., 2012; Giske et al., 2008; Kadetoff & Kosek, 2010; Light et al., 2009; Torpy et al., 2000). As sample extraction did not necessarily take place at the same time of day across studies, these discrepancies may be due to diurnal variations in catecholamine levels. Alternatively, they may be due to the state of physical and/or mental stress of the participants at the moment of the sample extraction as catecholamines are implicated in the body's stress response.

We found significantly lower dopamine levels in women with FM in comparison with controls. Likewise, in a previous study, Riva, Mork, Westgaard, Okkenhaug Johansen, and Lundberg (2012) found reduced urinary dopamine levels in patients with FM versus healthy controls. In contrast, Torpy et al. (2000) reported no differences in plasma dopamine levels in subjects with FM compared with the control group. These conflicting findings may be due to the different methodologies used to assess catecholamine levels. Torpy et al. used electrochemical detection, while we used fluorimetric, which is the more sensitive of the two methods. In addition, Torpy et al. analyzed plasma samples of seven patients with FM and seven controls. This low sample size may have masked differences in catecholamine levels between the two groups. With our higher

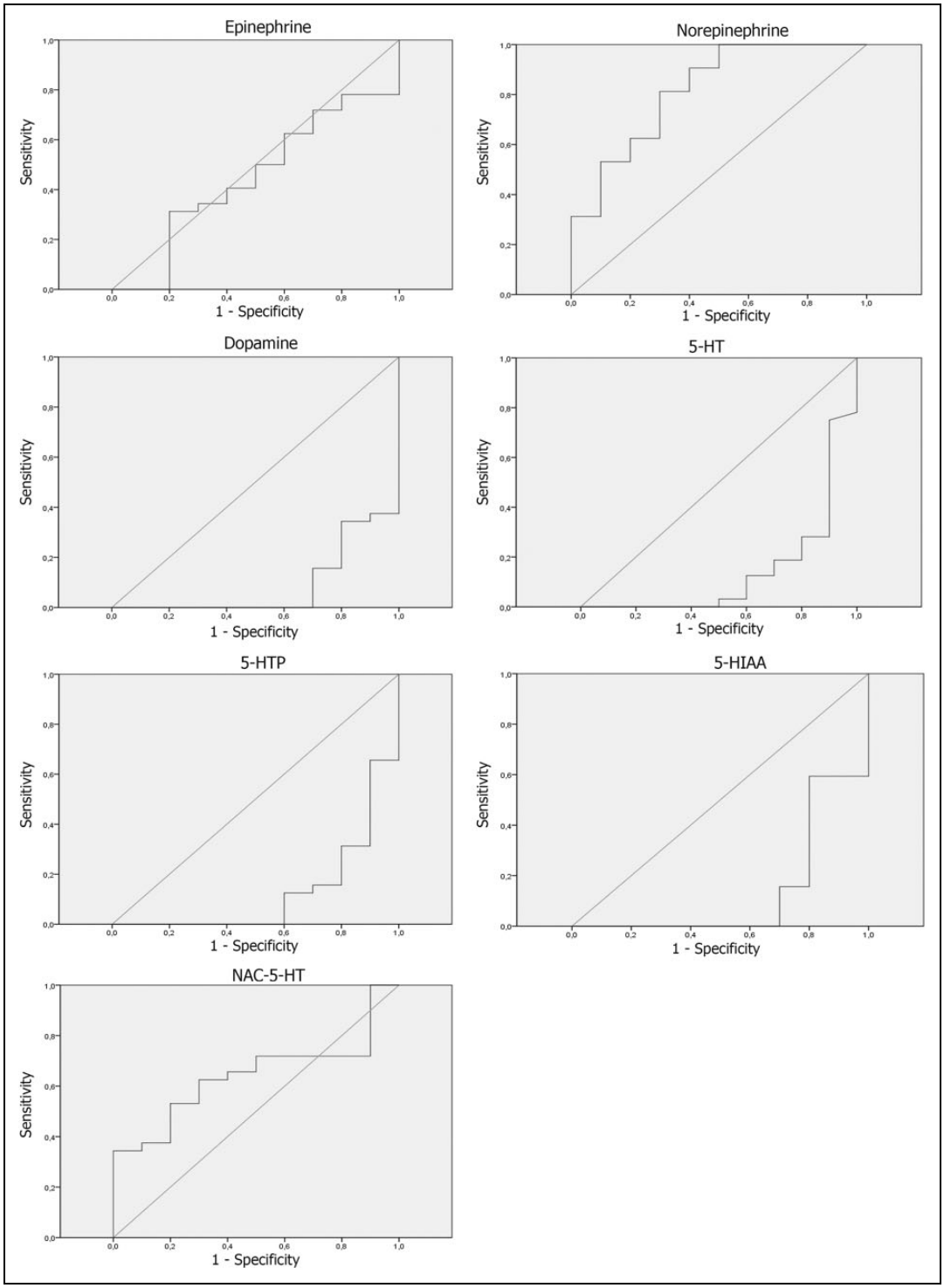

Figure 1. Receiver operating characteristic curves for plasma levels of catecholamines and indolamines among patients with fibromyalgia ($n = 35$) and healthy controls ($n = 12$). 5-HIAA = 5-hydroxyindolacetic acid; 5-HT = serotonin; 5-HTTP = 5-hydroxytryptophan; Nac-5-HT = *N*-acetyl-5-hydroxytryptamine.

number of participants, our results should be more consistent at the statistical level than those of Torpy et al.

Dopamine, the precursor of norepinephrine and epinephrine is the first catecholamine synthesized from L-3,4-dihydroxyphenylalanine (L-DOPA) by the enzyme L-DOPA decarboxylase. Low levels of L-DOPA might explain the reduced dopamine values we detected in patients with FM in the present study. Along with these lines, positron emission tomography revealed reductions in L-DOPA uptake in several brain regions of patients with FM in a previous study,

indicating reduced dopamine metabolism (Wood, Patterson, et al., 2007). On the other hand, the enzyme L-DOPA decarboxylase requires pyridoxal phosphate (PLP), a form of vitamin B6, as a cofactor. Researchers have reported that a PLP deficit is associated with a physical foot disorder known as Morton's foot syndrome (Nichols & Gaiteri, 2014), which, in turn, has been associated with FM (Starlanyl & Copeland, 2001). Moreover, several studies found that a deficiency in PLP was associated with increased oxidative stress (Sakakeeny et al., 2012; Shen, Lai, Mattei, Ordovas, & Tucker, 2010), which has been extensively related to the pathophysiology of FM. We previously reported that patients with FM showed significantly increased levels of markers of oxidative stress in comparison with healthy volunteers (La Rubia et al., 2013). Although we did not find significant correlations between dopamine levels and pain in the present study in participants with FM, other authors have reported that these patients have an abnormal dopamine response to pain, suggesting that this altered response may contribute to the widespread pain that occurs in FM (Wood, Schweinhardt, et al., 2007).

We also found significantly higher levels of norepinephrine in women with FM in comparison with controls in the present study, a finding that is in agreement with those of other studies (Bote et al., 2012; Torpy et al., 2000). However, other authors reported lower (Kadetoff & Kosek, 2010; Light et al., 2009) and similar (Giske et al., 2008) plasma levels of norepinephrine in patients with FM in comparison with healthy subjects. The enzyme that catalyzes the synthesis of norepinephrine from dopamine is dopamine-β-hydroxylase, which may be overactivated or upregulated in patients with FM given the altered levels of dopamine and norepinephrine we have found in these patients. Chronic stress induced upregulation of dopamine β-hydroxylase expression in the rat brain in one study, which may increase norepinephrine synthesis (Fan, Chen, Li, & Zhu, 2013). Chronic stress can lead to the development of several psychiatric disorders such as depression, which is common in patients with FM, in part as a result of reduced 5-HT levels (van Praag, 2005), which we also found in the women with FM in the present study. Moreover, a polymorphism in the gene-encoding dopamine β-hydroxylase has been associated with higher plasma activity of this enzyme in patients with mood or anxiety disorders (Cubells et al., 1998), which are frequently suffered by patients with FM (Penrod et al., 2004). Dopamine β-hydroxylase is a copper-containing tetrameric monooxygenase. It specifically binds 8 mol of copper per tetramer, which is required for maximal activity (Ash, Papadopoulos, Colombo, & Villafranca, 1984). We previously reported that copper levels were higher in patients with FM in comparison with healthy subjects (La Rubia et al., 2013), suggesting that such a high level may allow maximal activity of dopamine-β-hydroxylase in these patients, thereby decreasing the levels of the substrate (dopamine) and increasing those of the product (norepinephrine).

The catecholamines pathway is extremely complex and involves many enzymes that must be highly regulated. Plasma catecholamines are degraded either by deamination by monoamine oxidase (MAO) or by methylation by catechol-O-methyltransferase (COMT). Both enzymes are widely distributed in both the central nervous system and peripheral tissues and regulate the plasma levels of catecholamines (Fowler, Logan, Volkow, & Wang, 2005; Myöhänen & Männistö, 2010). We can eliminate the possibility that COMT was altered or dysregulated in patients with FM in the present study because the data showed a different tendency for each catecholamine in patients versus controls. Since COMT presents a similar preference for all catecholamines, its alteration should cause similar changes in plasma levels of dopamine, norepinephrine, and epinephrine, which is not what we found.

The MAO family comprises two isoenzymes, termed MAO-A and MAO-B. MAO-A preferentially degrades norepinephrine, while both isoforms metabolize dopamine. The high levels of norepinephrine we found in patients with FM in the present study might, then, be caused by hypoactivity of MAO-A, which would increase the bioavailability of norepinephrine considerably. Along with these lines, in a study performed on caregivers for relatives with dementia researchers found that a polymorphism of MAO-A associated with less transcriptional activity was related to increased depression and poorer sleep quality (Brummett et al., 2007), symptoms characteristic of patients suffering from FM. Furthermore, in another study, several MAO-A polymorphisms were associated with postsurgical pain in females undergoing oral surgery (Kim, Lee, Rowan, Brahim, & Dionne, 2006), and pain is the primary symptom of patients with FM. The genes that encode MAO are located on the X chromosome, meaning that the polymorphisms likely occur with disproportionate frequency in women compared with men, which might help to explain the higher prevalence of FM in women than in men (Jones et al., 2015). Recently, the European League Against Rheumatism revised the recommendations for managing FM and no longer recommends several pharmacological therapies such as MAO-A inhibitors because of lack of efficacy and a high risk of side effects (Macfarlane et al., 2017). The fact that MAO-A inhibitors do not improve symptoms supports our hypothesis of low MAO-A activity in FM. This hypothetically low activity of MAO-A in patients with FM might also increase the bioavailability of the other neurotransmitters it degrades such as dopamine. However, as mentioned above, dopamine can also be metabolized by MAO-B. In fact, a deficiency in one of these isoenzymes can cause the other to alter its activity to counter that deficiency (Chen, Holschneider, Wu, Rebrin, & Shih, 2004). Thus, if MAO-A was hypoactive in patients with FM, increasing dopamine values, MAO-B could hyperactivate to counteract such an effect and diminish levels of dopamine below normal values, which is what we found in patients with FM in the present study.

The high levels of norepinephrine we found in women with FM in the present study did not result in increased epinephrine values. Several prior studies also found that epinephrine values were similar in subjects with FM and controls (Giske et al., 2008; Light et al., 2009; Torpy et al., 2000), while other authors reported lower plasma levels of epinephrine in patients than in healthy subjects (Kadetoff & Kosek, 2010). The enzyme

phenylethanolamine-*N*-methyltransferase (PNMT) is responsible for synthesizing epinephrine from norepinephrine. Hypoactivity of this enzyme could lead to normal levels of epinephrine despite the high norepinephrine values we detected in patients with FM in the present study. Burke, Chung, Nakra, Grossberg, and Joh (1987) found that PNMT activity was decreased in subjects suffering from Alzheimer's disease, a pathology that, like FM, is related to oxidative stress. MAO-A is also indirectly involved in the synthesis of epinephrine from norepinephrine, as it degrades norepinephrine to the intermediary metabolite epinine that can be further transformed into epinephrine. In this way, the previously suggested low activity of MAO-A in patients with FM may also help to explain the normal levels of epinephrine found in these patients despite the high norepinephrine values that they presented.

It can take up to 5 years to diagnose FM. Thus, it is imperative to find markers that help in both the diagnosis and treatment of this condition (Brown et al., 2012). Our ROC curves showed that almost 90% of subjects who have norepinephrine values higher than 694.69 pg/ml can be diagnosed with FM with high sensitivity and specificity, suggesting that plasma levels of norepinephrine may be a good biomarker for FM diagnosis. In addition, in patients with FM we found that plasma levels of norepinephrine negatively correlated with score on the PCS-12, suggesting that high norepinephrine values are related to worse health status in these patients. Also, norepinephrine levels positively correlated with systolic and diastolic BP in subjects with FM (in the case of diastolic BP, the correlation only approached statistical significance). Along with these lines, researchers have reported that plasma norepinephrine predicts subsequent BP elevation in healthy men (Masuo, Kawaguchi, Mikami, Ogihara, & Tuck, 2003). However, we failed to find any significant association between norepinephrine levels and BP in the healthy women. Although BP was higher in our FM patients in comparison with controls, the difference was not statistically significant, possibly due to the small sample size. These results suggest that peripheral norepinephrine is involved in modulating both physical health status and BP in patients suffering from FM and, further, that this catecholamine likely contributes to the physiopathology of this syndrome. Another characteristic symptom of subjects with FM is pain, which researchers have found increases after norepinephrine injection in patients with FM in comparison with healthy people (Martinez-Lavin et al., 2002). It is possible that the limited sample size of the present study inhibited our ability to find significant correlations with other FM symptoms such as pain or functional capacity.

With regard to the indolamines, patients with FM in the present study showed lower levels of 5-HT and the intermediary metabolites 5-HIAA and 5-HTP than those of healthy women, suggesting that FM is related to disturbed 5-HT metabolism. Contrary to the conflicting results published on catecholamine values in patients with FM, the levels of 5-HT reported in previous studies of FM have largely agreed with our findings in the present study. Specifically, most of the available studies found reduced circulating levels of 5-HT in patients with FM compared with the control group (Bote et al., 2012; Russell, Michalek, et al., 1992; Stratz et al., 1993; Wolfe, Russell, Vipraio, Ross, & Anderson, 1997). Similarly, researchers have also reported lower levels of 5-HIAA in the cerebrospinal fluid of patients with FM than in healthy subjects (Legangneux et al., 2001; Russell, Vaerøy, Javors, & Nyberg, 1992). Since researchers have observed low levels of 5-HT in subjects with FM, its precursor, 5-HTP, has been used in the treatment of this syndrome, leading to significant improvements in pain, fatigue, number of tender points, quality of sleep, and anxiety in these patients (Puttini & Caruso, 1992).

The low levels of these products of the indolamine pathway found in patients with FM would be explained if the levels of the precursor of all of them, the amino acid tryptophan, were also reduced in FM patients. Researchers have detected significantly decreased plasma levels of tryptophan in subjects with FM in comparison with controls (Russell, Michalek, Vipraio, Fletcher, & Wall, 1989). Other authors have also reported low tryptophan values in these patients versus controls, but the differences only approached statistical significance ($p = .051$; Maes et al., 2000). Alternatively, the low levels of 5-HT, 5-HIAA, and 5-HTP we detected in the women with FM in the present study could indicate that the levels of the enzymes involved in their metabolism are altered in these patients, though there is no information available in the literature on possible alterations of these enzymes in FM. 5-HT is synthesized from 5-HTP by the enzyme 5-HTP decarboxylase. In turn, 5-HTP is synthesized from the amino acid tryptophan by tryptophan hydroxylase. Finally, 5-HT is metabolized mainly to 5-HIAA in two reactions: a first oxidation by MAO to the corresponding aldehyde and a second oxidation by aldehyde dehydrogenase to 5-HIAA. MAO-A has a much greater affinity for 5-HT than does MAO-B. As suggested above, patients with FM may have low MAO-A activity, which may increase the bioavailability of 5-HT. However, under conditions of MAO-A hypoactivity, MAO-B can deaminate its nonpreferred substrate to counteract such low activity (Chen et al., 2004), decreasing the levels of 5-HT below baseline values. In addition, 5-HTP decarboxylase is a vitamin B6–dependent enzyme. Therefore, a deficit of this vitamin in patients with FM might diminish the activity of 5-HTP decarboxylase and, consequently, 5-HT levels. As mentioned above, researchers have reported that a deficit of a form of vitamin B6 was associated with Morton's foot syndrome (Nichols & Gaiteri, 2014), which has demonstrated an association with FM (Starlanyl & Copeland, 2001). Unlike in other studies, we failed to find significant associations between levels of 5-HT and clinical features in patients with FM in the present study, a finding that might be attributable to the limited number of subjects involved. However, previous researchers have reported that circulating levels of 5-HT were related to pain (Ernberg et al., 2000; Russell, Michalek, et al., 1992), tender points (Stratz et al., 1993), and pressure tenderness (Stratz et al., 1993) in patients with FM. Our results in the present study suggest that dysregulation of indolamine metabolism, due to either a deficiency in tryptophan levels or the alteration of any

of the enzymes involved in this pathway, may play a pathophysiologic role in FM.

We also found that Nac-5-HT levels did not differ significantly between subjects with FM and controls. Nac-5-HT is an intermediate in the pathway by which melatonin is synthesized from 5-HT. It is produced from 5-HT in a reaction catalyzed by the enzyme serotonin *N*-acetyltransferase and is converted to melatonin by acetylserotonin O-methyltransferase. Based on the normal values of NAC-5-HT, we found in the FM patients in the present study, one would expect that melatonin levels were also similar between patients with FM and controls, as several authors have previously described (Klerman, Goldenberg, Brown, Maliszewski, & Adler, 2001; Press et al., 1998; Senel, Baygutalp, Baykal, Erdal, & Ugur, 2013).

The main limitation of the present study is the small sample size, which makes our findings preliminary and necessitates additional studies to verify the results. It is our intention to corroborate these results by increasing the number of participants and analyzing the activity of the main enzymes involved in the synthetic pathway of catecholamines and indolamines. Another limitation is that we did not control for the menstrual cycle phase of the participants, which could affect catecholamine and indolamine levels. However, most of the women (85.1%) were in similar conditions (70.2% were menopausal and 14.9% were premenopausal), which should have, at least partially, mitigated possible variations in the levels of these molecules due to the menstrual cycle phase.

Conclusions

Our findings in the present study show evidence of altered regulation of the metabolism of catecholamines and indolamines in patients with FM, which may contribute to the etiology of this syndrome. We found, for the first time, that norepinephrine levels above 694.69 pg/ml may be an accurate predictor of FM, which may greatly help in the diagnosis and treatment of this syndrome. We also found that high norepinephrine levels were related to worse physical health status in patients with FM. These data together suggest that the dysregulation of norepinephrine may be involved in the physiopathology of FM. These results provide insight into the physiopathologic mechanisms of FM that may ultimately help in the diagnosis, management, and treatment of patients with FM.

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Author Contributions

Rus, A contributed to conception and design contributed to acquisition, analysis, and interpretation drafted manuscript critically revised manuscript gave final approval agrees to be accountable for all aspects of work ensuring integrity and accuracy. Molina, F contributed to conception and design contributed to acquisition, analysis, and interpretation drafted manuscript critically revised manuscript gave final approval agrees to be accountable for all aspects of work ensuring integrity and accuracy. Del Moral, ML contributed to conception and design critically revised manuscript gave final approval agrees to be accountable for all aspects of work ensuring integrity and accuracy. Ramírez-Expósito, MJ contributed to acquisition critically revised manuscript gave final approval agrees to be accountable for all aspects of work ensuring integrity and accuracy. Martínez-Martos, JM contributed to analysis and interpretation critically revised manuscript gave final approval agrees to be accountable for all aspects of work ensuring integrity and accuracy.

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