Effect of menopause on circulating amino acid concentrations in women with fibromyalgia and healthy women

Alma Rus[a,b] (mrus@ugr.es), Bárbara Coca-Guzmán[a,c] (barbaracg@correo.ugr.es), Francisco Molina[b,c] (fjmolina@ugr.es), María Correa-Rodríguez[b,d]* (macoro@ugr.es), José Manuel Martínez-Martos[e] (jmmartos@ujaen.es), María Jesús Ramírez-Expósito[e] (mramirez@ujaen.es), María Encarnación Aguilar-Ferrándiz[b,c] (e_aguilar@ugr.es)

[a] Department of Cell Biology, University of Granada, Avenida de la Fuentenueva, s/n, 18071, Granada, Spain.

[b] Instituto de Investigación Biosanitaria ibs.GRANADA, 18012 Granada, Spain.

[c] Department of Physical Therapy, Faculty of Health Sciences, University of Granada, Avenida de la Ilustración, 60, 18016, Granada, Spain. Instituto de Investigación Biosanitaria ibs.GRANADA, 18012 Granada, Spain.

[d] Department of Nursing, Faculty of Health Sciences, University of Granada, Avenida de la Ilustración, 60, 18016, Granada, Spain. Instituto de Investigación Biosanitaria ibs.GRANADA, 18012 Granada, Spain.

[e] Department of Health Science, University of Jaén, Campus Las Lagunillas s/n, 23071, Jaén, Spain.

*Corresponding author

Abstract

Objectives. Fibromyalgia is a complex syndrome that appears more frequently during menopause. There are no previous studies investigating the effect of menopause on amino acids in fibromyalgia. Therefore, we have examined serum amino acid concentrations in premenopausal and postmenopausal women with fibromyalgia and healthy women.

Study design. A case-control study was carried out in 28 healthy premenopausal and 46 postmenopausal women and in 16 premenopausal and 52 postmenopausal women with fibromyalgia. This study adheres to STROBE guideline.

Main outcome measures. Amino acid content was assayed by high performance liquid chromatography.

Results. Significant differences were found in concentrations of several amino acids (aspartic acid, glutamic acid, histidine, glycine, alanine, leucine, and taurine) between healthy premenopausal women and premenopausal women with fibromyalgia and between healthy postmenopausal women and postmenopausal women with fibromyalgia. Concentrations of other amino acids (aminoadipic acid, asparagine, threonine, arginine, 5-methyl-histidine, valine, methionine, isoleucine, phenylalanine, ornithine, branched-chain amino acids, large neutral amino acids, essential amino acids, non-essential amino acids, basic amino acids, and arginine/ornithine ratio) were found to be altered between healthy postmenopausal women and postmenopausal women with fibromyalgia, but not between healthy premenopausal women and premenopausal women with fibromyalgia. No significant differences were found in serum amino acid concentrations between healthy premenopausal and postmenopausal women or between premenopausal and postmenopausal women with fibromyalgia.

Conclusions. Our results show, for the first time, that the association between menopause and FM may increase the risk of metabolic disorders by disrupting amino acid homeostasis to a greater extent than menopause or fibromyalgia alone.

Keywords: amino acid, fibromyalgia, menopause.

1. Introduction

Fibromyalgia (FM) is a chronic widespread pain syndrome characterized by symptoms such as idiopathic musculoskeletal pain, fatigue, sleep disturbances, and anxiety [1]. FM affects approximately 2% to 4% of the general population [2]. Patients with FM are typically 50–64 years old women [3,4]. Although its physiopathology is not yet fully understood, recent investigations have pointed to a disorder of pain regulation and central sensitization [5], mediated by altered concentrations of neurotransmitters such as catecholamines, serotonin [6], and several amino acids (AAs) as glutamic acid or γ-aminobutyric acid (GABA) [7]. In this line, the main symptoms of FM have been reported to be associated to dysfunction of neurotransmitters. Thus, increased pain perception, fatigue, sleep disturbances, and depressive symptoms have been related to alterations in the function of several neurotransmitters [8].

In addition to their main role for the synthesis of tissue proteins, AAs play important roles in neurotransmission, regulating gene expression, cell signalling, nutrient metabolism, hormone secretion, and immune response, among others [9]. Many AAs participate in neurotransmission, either as neurotransmitters, precursors of neurotransmitters (such as catecholamines or serotonin) or neuromodulators [10]. Glutamic acid and GABA are the main excitatory and inhibitory neurotransmitters of the central nervous system (CNS), respectively. These two AAs have been reported to play an important role in the processing of pain [11,12], the main symptom of patients diagnosed with FM. In fact, the dysregulation between glutamic acid and GABA has been proposed as a mechanism that may trigger chronic pain [12].

On the other hand, FM appears more frequently in postmenopausal women [13]. The Stages of Reproductive Aging Workshop (STRAW+10) criteria divided the adult

woman life into 3 phases: reproductive, menopausal transition, and postmenopause [14]. The menopausal transition is characterized, in an early phase, by variability in menstrual cycle length and, subsequently, by onset of skipped cycles or amenorrhea of at least 60 days [14]. The World Health Organization (WHO) defines menopause (or the final menstrual period) as the cessation of menstruation, determined retrospectively after 12 consecutive months of amenorrhea, without pathological causes. The postmenopause phase is divided into early and late postmenopause [14]. The menopausal transition and early postmenopause phases are characterized by musculoskeletal pain, arthralgia, fatigue, sleep disturbances, mood swings, anxiety, headaches, difficulty in concentrating, vasomotor symptoms, vaginal dryness, decreased libido, and weight gain [15]. The characteristic symptoms of these phases have been called “climacteric syndrome” by some authors [15]. Due to the similarity between symptoms of both FM and “climacteric syndrome”, it has even been hypothesized that FM could be part of the “climacteric syndrome”, in which an alteration of neurotransmission occurs [3,16]. In line, it has been reported that menopause increased the severity of pain and other symptoms in patients with FM [17]. Other authors found that early onset of menopause may contribute to pain hypersensitivity in chronic musculoskeletal pain syndromes such as FM [18].

The studies investigating the role of menopause in FM are very scarce and they were only focused on pain variables. To the best of our knowledge, no previous work has investigated the effect of menopause on biological molecules such as AAs in women with FM. Knowing the possible differences that occur during menopause in women with FM compared to healthy women will undoubtedly help us to increase our knowledge about the pathophysiology of this complex syndrome. Therefore, our aim

was to evaluate a broad range of serum AA concentrations in premenopausal and postmenopausal women with FM and in healthy premenopausal and postmenopausal women.

2. Methods

2.1. Type of study

This case-control study was carried out in accordance with the Declaration of Helsinki of the World Medical Association (WMA). The study was approved by the Granada Provincial Research Ethics Committee (Junta de Andalucía, Spain), with approval number 1797-N-17. This study adheres to the STROBE guideline.

2.2. Determination of sample size

Sample size was determined using Ene 3.0 software (GlaxoSmithKline, Rockbille, MA, USA). To achieve a power (1 – β error) of 0.80, taking into account a significance level (α error) of 0.05, and based on the results of plasma alanine concentration in patients with FM [19], it is necessary to include at least 10 subjects per group in the study.

2.3. Participants

The participants were distributed into 4 groups: healthy premenopausal women (group 1), healthy postmenopausal women (group 2), premenopausal women with FM (group 3), and postmenopausal women with FM (group 4). Premenopausal women reported regular periods, and postmenopausal women reported no periods for at least 12 months. Women with FM were contacted both via AGRAFIM (Association of Fibromyalgia of Granada, Spain), and AFIXA (Association of Fibromyalgia of Jaén, Spain). Healthy women were recruited from relatives and friends of patients with FM and from the staff of the University of Granada. All participants provided written informed consent and did not receive financial incentive.

The patients had previously been diagnosed with FM by a professional rheumatologist of the Public Health System of Andalucía (Spain) and met the 2010 American College of Rheumatology (ACR) criteria for FM. The inclusion criteria, for both patients and controls, were being a woman and being between 30 and 75 years of age. The exclusion criteria for all participants were presence of any chronic disease (e.g. diabetes mellitus, cancer, hypertension, or cardiac, digestive, renal, pulmonary, hepatic or rheumatic disease), grade II obesity (body mass index (BMI) $\geqslant$ 35 kg/m^2), severe physical disability, psychiatric illness, previous history of surgery, pregnancy or lactation, and treatment with vasoactive drugs, anticoagulants, corticosteroids, or agonist / antagonist opioid receptors. No women were taking hormone replacement therapy or hormonal contraceptives.

2.4. Demographic and clinical data of participants

Each participant provided demographic and clinical data by means of interviews and questionnaires carried out by the same specialist in our laboratories of the Faculty of Health Sciences of the Universities of Granada and Jaén (Spain) between January and April 2022. All the questionnaires were completed by patients with FM and by controls with the exception of the Revised Fibromyalgia Impact Questionnaire (FIQ-R) that was only completed by the patients.

The severity of FM was assessed by the Spanish version of the FIQ-R, which has an internal consistency (Cronbach's alpha) of 0.91 [20]. The questionnaire consists of 21 items and the total score ranges from 0 to 100, assessing physical impairment, number of days feeling good, work missed, ability to do work, pain, fatigue, restfulness,

stiffness, anxiety, and depressive symptoms. The total score came from the sum of all subscales (activity level, overall impact, and intensity of symptoms). The degree of FM symptoms according to the FIQ-R is established by the following cut-off points: FIQ-R⩽30 reflects no severity, FIQ-R>30 and ⩽45 reflects mild severity, FIQ-R>46 and ⩽65 reflects moderate severity, and FIQ-R>65 reflects high severity.

Self-reported musculoskeletal pain was measured according to a visual analogue scale (VAS) whose score ranges from 0 to 100, with higher values reflecting more pain. This scale has shown a high sensibility and specificity in the FM population [21].

Fatigue was evaluated by the Spanish version of the Multidimensional Fatigue Inventory (MFI) [22], which consists of 20 items and has a potential score range from 20 to 100. The questionnaire contains five dimensions: general fatigue, physical fatigue, reduced activity, reduced motivation, and mental fatigue. The scores per dimension run from 1 to 5 points, with higher scores indicating more fatigue. The Spanish version of the MFI has shown high internal consistency, with a Cronbach's alpha of 0.93 [23].

The anxiety-related symptoms were evaluated using a Spanish version of the Beck Anxiety Inventory (BAI) [24], which has a Cronbach's alpha of 0.93 [25]. The BAI consists of 21 items, and each item is scored from 0 points (nothing anxiety) to 3 points (a lot of anxiety). The total score ranges from 0 to 63 points, with higher score indicating increased anxiety.

Sleep quality was evaluated using the Spanish version of the Pittsburgh Sleep Quality Index (PSQI), which has an internal consistency (Cronbach's alpha) of 0.805 [26]. The

questionnaire consists of 24 items and has seven subscales: subjective quality, sleep latency, sleep duration, habitual sleep efficacy, sleep perturbations, use of night medication, and daily dysfunction. Each subscale is scored from 0 (no problems) to 3 points (severe problems). The total score varies in a range from 0 to 21 points, and higher score indicates poorer sleep quality.

2.5. Blood collection

To avoid potential circadian variations in AA concentrations, blood samples were collected at the same time of day. Venous blood was taken after an overnight fast by the same practitioner from the antecubital vein into an anticoagulant-free tube after the participants completed the questionnaires. Blood was allowed to clot for 30 min at room temperature. The tube was then centrifuged at 3500 rpm for 5 min at 4°C to obtain serum samples.

2.6. Determination of serum AA concentrations

AA content was assayed by High Performance Liquid Chromatography (HPLC) coupled to a fluorescence detection system as we previously described [27]. Briefly, serum samples were deproteinated by ultrafiltration, and the deproteinated serum was precolumn derivatized with OPA reagent (o-phthaldialdehyde in borate buffer pH 9.5 containing 3-mercaptopropionic acid) and injected through a refrigerated Perkin-Elmer Series 200 automatic sample injector into a 150 x 3.9 mm Waters Resolve 5µ C-18 column. The fluorescence detector (Perkin-Elmer Series 200a) was set at an excitation wavelength of 340 nm and an emission wavelength of 420 nm. Data were processed with the TotalChrom WorkStation ver. 6.3.1 software (Perkin-Elmer). Concentrations were expressed as picomoles of AA per microliter (pmoles/µL).

2.7. Calculations

The abbreviations for each AA are indicated in Table 2. Valine, leucine, and isoleucine were summed as branched-chain AAs (BCAAs). BCAAs plus tyrosine and phenylalanine were summed as large neutral AAs (LNAAs). BCAAs along with phenylalanine, methionine, threonine, lysine, and histidine were summed as essential AAs (EAAs). Alanine, glycine, serine, glutamine, arginine, taurine, glutamic acid, asparragine, aspartic acid, ornithine, and citrulline were summed as nonessential AAs (NEAAs). Arginine, ornithine, lysine, and histidine were summed as basic AAs (BAAs).

The GABR ratio (global arginine bioavailability ratio) was calculated as the ratio between arginine concentration and the sum of the concentrations of ornithine and citrulline [28]. It is an indicator of the global bioavailability of arginine, which has been reported to be more reliable than arginine concentration [29]. Low GABR ratio has been related to cardiovascular disease, diabetes, and pulmonary hypertension [30]. The arginine/ornithine ratio may reflect arginase activity, and it has been associated to mortality in sickle cell disease patients [30]. The TSM ratio was calculated as the ratio between a hundred times the taurine concentration, and the product of the concentrations of serine and methionine. It is an indicator of the AAs involved in transmethylation processes, which are altered in diseases such as Alzheimer's disease [31] and psychoses [32].

2.8. Statistical analysis

The statistical analysis of the data was performed using the statistical package IBM SPSS Statistics 24 for Windows (SPSS Inc, Chicago, IL). Data were expressed as mean

± standard deviation (SD). The data that followed a normal distribution (tested with Kolmogorov–Smirnov test) and the principle of homoscedasticity of variances (tested with Levene test) were tested by ANOVA (age, BMI, FIQ-R, MFI, citrulline, taurine, carnosine, and GABA). The statistical significance was established by applying an unpaired Student's t-test to compare differences between means. The data that did not follow a normal distribution or the principle of homoscedasticity were tested using the Kruskal Wallis test. The degree of statistical significance was established by applying the Mann-Whitney U test to compare differences between means. We set the level of statistical significance at $p < 0.05$.

3. Results

3.1. Participants

The demographic and clinical characteristics of the participants are shown in Table 1. Ninety-one women with FM and 89 healthy women were interested in participating in our study, but 23 patients and 15 controls were excluded for meeting any of the exclusion criteria. Finally, 68 women with FM (52 were postmenopausal and 16 were premenopausal) and 74 healthy women (46 were postmenopausal and 28 were premenopausal) participated in the study. As expected, there were statistically significant differences in age between healthy premenopausal women and healthy postmenopausal women ($p<0.001$) and between premenopausal women with FM and postmenopausal women with FM ($p<0.001$). There were no statistically significant differences between the study groups by BMI. FIQ-R scores of both premenopausal and postmenopausal women with FM reflected high severity of FM. Significant differences were found in VAS, MFI, BAI, and PSQI scores between healthy premenopausal women and premenopausal women with FM (all $p<0.001$) and between healthy postmenopausal women and postmenopausal women with FM (all $p<0.001$). There were no statistically significant differences in these questionnaire scores between healthy premenopausal and postmenopausal women or between premenopausal and postmenopausal women with FM.

3.2. Serum AA concentrations

Significant differences were found in the concentrations of several AAs (aspartic acid, glutamic acid, histidine, glycine, alanine, leucine, and taurine) between healthy premenopausal women and premenopausal women with FM ($p\leq0.025$; Table 2) and

between healthy postmenopausal women and postmenopausal women with FM ($p \leq 0.001$; Table 2). We also found significant differences in aminoadipic acid, asparagine, threonine, arginine, 5-methyl-histidine, valine, methionine, isoleucine, phenylalanine, ornithine, BCAAs, LNAAs, EAAs, NEAAs, BAAs, and arginine/ornithine ratio between healthy postmenopausal women and postmenopausal women with FM ($p \leq 0.046$; Tables 2 and 3), but not between healthy premenopausal women and premenopausal women with FM. There were no statistically significant differences in serum AA concentrations between healthy premenopausal and postmenopausal women or between premenopausal and postmenopausal women with FM (Tables 2 and 3).

4. Discussion

The prevalence of FM is higher during menopause. However, the possible association between FM and menopause has not been sufficiently investigated. We have assessed the serum AA profile in premenopausal and postmenopausal women with FM as well as in healthy premenopausal and postmenopausal woman. Results did not show statistically significant differences in serum AA concentrations between premenopausal women with FM and postmenopausal women with FM or between healthy premenopausal women and healthy postmenopausal women, suggesting that menopause may not alter serum AA concentrations, neither in women with FM nor in healthy women. Conversely, several studies have reported that menopause may modulate the circulating metabolome, including the AA profile, in healthy women. A recent study showed that plasma concentrations of ornithine, taurine, glutamine, lysine, carnitine, hydroxyproline, arginine, and valine were significantly higher in postmenopausal women than in premenopausal women [33]. Similarly, menopause has been associated with decreased serum glutamine concentration and increased leucine concentration [34]. In a meta-analysis with more than 10,000 women, postmenopausal women had significantly higher serum concentrations of glutamine, tyrosine, and isoleucine than premenopausal women [35]. The same authors also reported that menopause was associated with glycine concentration in a group of more than 3,000 women aged 40–55 years.

We have found that the concentrations of several AAs (aspartic acid, glutamic acid, histidine, glycine, alanine, leucine, and taurine) showed significant differences between healthy premenopausal women and premenopausal women with FM as well as between healthy postmenopausal women and postmenopausal women with FM, indicating that

this syndrome may be responsible for the altered serum AA pattern found in women with FM compared to healthy women. We have found higher concentrations of aspartic acid, glutamic acid, histidine, glycine, leucine, and taurine as well as lower alanine concentration in premenopausal women with FM than in healthy premenopausal women and in postmenopausal women with FM than in healthy postmenopausal women. To our knowledge, there are no previous works examining the effect of menopause on concentrations of AAs or other biological molecules in women with FM compared to healthy women with which to compare our results. However, circulating AA concentrations have been previously examined in patients with FM in comparison to controls, showing contradictory results [19,36–41]. This is probably attributable to several factors, such as the selected sample size, or a different recruitment of patients, since patients with FM usually have many comorbidities that could explain the diversity of results obtained in these studies. The oldest studies [19,36,40] showed no changes or lower concentrations of the AAs investigated in patients with FM vs. controls, while the more recent works [37–39,41] have shown increased concentrations of several AAs in patients with FM compared to healthy subjects, coinciding with our results. In this line, significantly increased serum concentrations of glutamic acid, aspartic acid, taurine, glycine, phenylalanine, methionine, ornithine, and BCAAs have been reported in women diagnosed with FM compared to healthy women [37]. In agreement with our results, higher plasma taurine concentration has been reported in women with FM in comparison to healthy women [41]. Similarly, another study found higher serum concentrations of glutamic acid, ornithine, arginine, and threonine in patients with FM compared to controls [38]. Coinciding with our data, lower alanine concentration has been reported in patients with FM than in controls [19,39,40]. In addition, we have recently reported that patients with FM showed higher serum concentrations of aspartic

acid, glutamic acid, aminoadipic acid, asparagine, histidine, 3-methyl-histidine, 5-methyl-histidine, glycine, threonine, taurine, tyrosine, valine, methionine, isoleucine, phenylalanine, leucine, lysine, ornithine, BCAAs, LNAAs, EAAs, NEAAs, BAAs, and GABR ratio than healthy subjects [39].

In contrast to our data of unchanged AA profile due to menopause in both FM and healthy women, our results also showed significant differences in serum concentrations of aminoadipic acid, asparagine, threonine, arginine, 5-methyl-histidine, valine, methionine, isoleucine, phenylalanine, ornithine, BCAAs, LNAAs, EAAs, NEAAs, BAAs, and arginine/ornithine ratio between healthy postmenopausal women and postmenopausal women with FM, but not between healthy premenopausal women and premenopausal women with FM. We found higher concentrations of all these AAs and AA groupings in postmenopausal women with FM than in healthy postmenopausal women. These results suggest that FM might not be the only factor altering serum AA concentrations in postmenopausal women with FM. In the present study, we can rule out that such alterations could be due to age or BMI, since there were no statistically significant correlations between age or BMI and any of these AAs in postmenopausal women with FM (data not shown). Therefore, our results suggest that menopause, together with FM, may alter AA concentrations to a greater extent than menopause or FM alone. These findings highlight the risk of future metabolic disorders caused by disrupted AA homeostasis in postmenopausal women with FM. The mechanisms by which the menopause-FM association may influence AA concentrations to a greater extent than menopause or FM alone are unknown. It is possible that female sex hormones at physiological concentrations of fertile age may help normalize AA homeostasis, so that their abrupt menopause-induced decrease, together with the

disorders caused by FM syndrome, would alter this homeostasis. The cessation of ovarian functions has been associated with significant metabolic alterations and increased susceptibility to several pathologies associated with ageing. In this line, a direct association has been reported between postmenopausal hormonal shift and glutamine and tyrosine concentrations in women transitioning from perimenopause to early postmenopause [34]. The same authors found that postmenopausal hormone therapy specifically influenced glycine metabolism [34]. Another study reported that estradiol was associated with increased concentrations of glutamate and GABA found in a region of rat hypothalamus [42]. It has also been found that estrogen receptor plays a role in the regulation of estrogen-induced AA transport in breast cancer cells [43]. In addition, it has been demonstrated that isoform alpha of the estrogen receptor may be activated by both estrogens and AAs [44]. Thus, as estrogen concentration decreases during menopause, AA concentrations could increase to compensate for the decrease in estrogen levels, as shown by our results, thereby trying to alleviate the adverse effects of their decline.

The main limitation of the present study is the small sample size, which makes our findings preliminary and requires additional studies to verify the results. However, to our knowledge, this is the first study investigating the serum AA profile in premenopausal and postmenopausal women with FM and in healthy premenopausal and postmenopausal woman.

5. Conclusions

Our results suggest that the menopause-FM association may contribute to future metabolic disorders by altering AA concentrations to a greater extent than menopause or FM alone.

6. Acknowledgments

Authors would like to thank AGRAFIM (Association of Fibromyalgia of Granada, Spain) and AFIXA (Association of Fibromyalgia of Jaén, Spain) for participating in this study. This article is part of the PhD thesis developed by Bárbara Coca Guzmán, who is included in the Official PhD Program of Biomedicine from the University of Granada, Spain.

7. References

[1] V. Giorgi, S. Sirotti, M.E. Romano, D. Marotto, J.N. Ablin, F. Salaffi, P. Sarzi-Puttini, Fibromyalgia: one year in review 2022, Clin. Exp. Rheumatol. 40 (2022) 1065–1072. https://doi.org/10.55563/clinexprheumatol/if9gk2.

[2] G.T. Jones, F. Atzeni, M. Beasley, E. Flüß, P. Sarzi-Puttini, G.J. Macfarlane, The prevalence of fibromyalgia in the general population: a comparison of the american college of rheumatology 1990, 2010, and modified 2010 classification criteria, Arthritis Rheumatol. 67 (2015) 568–575. https://doi.org/10.1002/art.38905.

[3] J.E. Blümel, S. Palacios, D. Legorreta, M.S. Vallejo, S. Sarra, Is fibromyalgia part of the climacteric syndrome?, Maturitas 73 (2012) 87–93. https://doi.org/10.1016/j.maturitas.2012.06.001.

[4] M. Topbas, H. Cakirbay, H. Gulec, E. Akgol, I. Ak, G. Can, The prevalence of fibromyalgia in women aged 20-64 in Turkey, Scand. J. Rheumatol. 34 (2005) 140–144.

[5] M.J. Bair, E.E. Krebs, Fibromyalgia, Ann. Intern. Med. 172 (2020) ITC33. https://doi.org/10.7326/AITC202003030.

[6] A. Rus, F. Molina, M.L. Del Moral, M.J. Ramírez-Expósito, J.M. Martínez-Martos, Catecholamine and indolamine pathway: a case–control study in fibromyalgia, Biol. Res. Nurs. 20 (2018) 577–586. https://doi.org/10.1177/1099800418787672.

[7] S. Chinn, W. Caldwell, K. Gritsenko, Fibromyalgia pathogenesis and treatment options update, Curr. Pain Headache Rep. 20 (2016) 25. https://doi.org/10.1007/s11916-016-0556-x.

[8] S. Becker, P. Schweinhardt, Dysfunctional neurotransmitter systems in fibromyalgia, their role in central stress circuitry and pharmacological actions on these systems, Pain Res. Treat. 2012 (2012) 1–10. https://doi.org/10.1155/2012/741746.

[9] G. Wu, Functional amino acids in nutrition and health, Amino Acids 45 (2013) 407–411. https://doi.org/10.1007/s00726-013-1500-6.

[10] R. Dalangin, A. Kim, R.E. Campbell, The role of amino acids in neurotransmission and fluorescent tools for their detection, Int. J. Mol. Sci. 21 (2020) 6197. https://doi.org/10.3390/ijms21176197.

[11] C. Benson, K. Mifflin, B. Kerr, S.J.B. Jesudasan, S. Dursun, G. Baker, Biogenic amines and the amino acids gaba and glutamate: relationships with pain and depression, in: D.P. Finn, B.E. Leonard (Eds.), Mod. Trends Psychiatry, S. Karger AG, 2015, pp. 67–79. https://doi.org/10.1159/000435933.

[12] A.L. Peek, T. Rebbeck, N.Aj. Puts, J. Watson, M.-E.R. Aguila, A.M. Leaver, Brain GABA and glutamate levels across pain conditions: A systematic literature review and meta-analysis of 1H-MRS studies using the MRS-Q quality assessment tool, NeuroImage 210 (2020) 116532. https://doi.org/10.1016/j.neuroimage.2020.116532.

[13] S. Carranza-Lira, I.B. Villalobos Hernandez, Prevalence of fibromyalgia in premenopausal and postmenopausal women and its relation to climacteric symptoms, Prz. Menopauzalny 13 (2014) 169–173. https://doi.org/10.5114/pm.2014.43819.

[14] S.D. Harlow, M. Gass, J.E. Hall, R. Lobo, P. Maki, R.W. Rebar, S. Sherman, P.M. Sluss, T.J. De Villiers, for the Straw +10 Collaborative Group, Executive summary of the Stages of Reproductive Aging Workshop +10: addressing the unfinished agenda of staging reproductive aging, Climacteric 15 (2012) 105–114. https://doi.org/10.3109/13697137.2011.650656.

[15] M. Sourouni, M. Zangger, L. Honermann, D. Foth, P. Stute, Assessment of the climacteric syndrome: a narrative review, Arch. Gynecol. Obstet. 304 (2021) 855–862. https://doi.org/10.1007/s00404-021-06139-y.

[16] I.B. Ozcivit, C.T. Erel, F. Durmusoglu, Can fibromyalgia be considered a characteristic symptom of climacterium?, Postgrad. Med. J. 99 (2023) 244–251. https://doi.org/10.1136/postgradmedj-2021-140336.

[17] O.N. Pamuk, N. Cakir, The variation in chronic widespread pain and other symptoms in fibromyalgia patients. The effects of menses and menopause, Clin. Exp. Rheumatol. 23 (2005) 778–782.

[18] M. Martínez-Jauand, C. Sitges, J. Femenia, I. Cifre, S. González, D. Chialvo, P. Montoya, Age-of-onset of menopause is associated with enhanced painful and non-painful sensitivity in fibromyalgia, Clin. Rheumatol. 32 (2013) 975–981. https://doi.org/10.1007/s10067-013-2212-8.

[19] L. Bazzichi, L. Palego, G. Giannaccini, A. Rossi, F. De Feo, C. Giacomelli, L. Betti, L. Giusti, G. Mascia, S. Bombardieri, A. Lucacchini, Altered amino acid

homeostasis in subjects affected by fibromyalgia, Clin. Biochem. 42 (2009) 1064–1070. https://doi.org/10.1016/j.clinbiochem.2009.02.025.

[20] M. Salgueiro, J.M. García-Leiva, J. Ballesteros, J. Hidalgo, R. Molina, E.P. Calandre, Validation of a spanish version of the revised fibromyalgia impact questionnaire (FIQR), Health Qual. Life Outcomes 11 (2013) 132. https://doi.org/10.1186/1477-7525-11-132.

[21] A.P. Marques, A. Assumpção, L.A. Matsutani, C.A.B. Pereira, L. Lage, Pain in fibromyalgia and discrimination power of the instruments: visual analog scale, dolorimetry and the mcgill pain questionnaire, Acta Reumatol. Port. 33 (2008) 345–351.

[22] D. Munguía-Izquierdo, V. Segura-Jiménez, D. Camiletti-Moirón, M. Pulido-Martos, I.C. Alvarez-Gallardo, A. Romero, V.A. Aparicio, A. Carbonell-Baeza, M. Delgado-Fernández, Multidimensional Fatigue Inventory: Spanish adaptation and psychometric properties for fibromyalgia patients. The Al-Andalus study, Clin. Exp. Rheumatol. 30 (2012) 94–102.

[23] A. Soriano-Maldonado, K. Amris, F.B. Ortega, V. Segura-Jiménez, F. Estévez-López, I.C. Álvarez-Gallardo, V.A. Aparicio, M. Delgado-Fernández, M. Henriksen, J.R. Ruiz, Association of different levels of depressive symptoms with symptomatology, overall disease severity, and quality of life in women with fibromyalgia, Qual. Life Res. 24 (2015) 2951–2957. https://doi.org/10.1007/s11136-015-1045-0.

[24] J. Sanz, M.E. Navarro, The psychometric properties of a Spanish version of the Beck Anxiety Inventory (Bai) in a university students sample, Ansiedad y Estrés 9 (2003) 59–84.

[25] I. Magán, J. Sanz, M.P. García-Vera, Psychometric properties of a Spanish version of the Beck Anxiety Inventory (Bai) in general population, Span. J. Psychol. 11 (2008) 626–640.

[26] F. Hita-Contreras, E. Martínez-López, P.A. Latorre-Román, F. Garrido, M.A. Santos, A. Martínez-Amat, Reliability and validity of the Spanish version of the Pittsburgh Sleep Quality Index (Psqi) in patients with fibromyalgia, Rheumatol. Int. 34 (2014) 929–936. https://doi.org/10.1007/s00296-014-2960-z.

[27] M.J. Ramírez-Expósito, M.D. Ruíz-Sanjuan, J.M. Martínez-Martos, Influence of dietary fats on circulating amino acid profile in experimental breast cancer, J. Clin. Mol. Med. 1 (2018) 1–5. https://doi.org/10.15761/JCMM.1000104.

[28] J.M. Martínez-Martos, M.E. Pulido-Navas, M.J. Ramírez-Expósito, Altered Plasma Global Arginine Bioavailability Ratio in Early-stage Alzheimer's Disease, Open Biomark. J. 8 (2018) 34–41. https://doi.org/10.2174/1875318301808010034.

[29] W.H.W. Tang, Z. Wang, L. Cho, D.M. Brennan, S.L. Hazen, Diminished Global Arginine Bioavailability and Increased Arginine Catabolism as Metabolic Profile of Increased Cardiovascular Risk, J. Am. Coll. Cardiol. 53 (2009) 2061–2067. https://doi.org/10.1016/j.jacc.2009.02.036.

[30] C.R. Morris, J. Hamilton-Reeves, R.G. Martindale, M. Sarav, J.B. Ochoa Gautier, Acquired Amino Acid Deficiencies: A Focus on Arginine and Glutamine, Nutr. Clin. Pract. 32 (2017) 30S–47S. https://doi.org/10.1177/0884533617691250.

[31] D. Fekkes, T.J.M. Van Der Cammen, C.P.M. Van Loon, C. Verschoor, F. Van Harskamp, I. De Koning, W.J. Schudel, L. Pepplinkhuizen, Abnormal amino acid metabolism in patients with early stage Alzheimer dementia, J. Neural Transm. 105 (1998) 287–294. https://doi.org/10.1007/s007020050058.

[32] D. Fekkes, L. Pepplinkhuizen, R. Verheij, J. Bruinvels, Abnormal plasma levels of serine, methionine, and taurine in transient acute polymorphic psychosis, Psychiatry Res. 51 (1994) 11–18. https://doi.org/10.1016/0165-1781(94)90043-4.

[33] K. Watanabe, M. Iida, S. Harada, S. Kato, K. Kuwabara, A. Kurihara, A. Takeuchi, D. Sugiyama, T. Okamura, A. Suzuki, K. Amano, A. Hirayama, M. Sugimoto, T. Soga, M. Tomita, Y. Kobayashi, K. Banno, D. Aoki, T. Takebayashi, Metabolic profiling of charged metabolites in association with menopausal status in Japanese community-dwelling midlife women: Tsuruoka Metabolomic Cohort Study, Maturitas 155 (2022) 54–62. https://doi.org/10.1016/j.maturitas.2021.10.004.

[34] J.E. Karppinen, T. Törmäkangas, U.M. Kujala, S. Sipilä, J. Laukkanen, P. Aukee, V. Kovanen, E.K. Laakkonen, Menopause modulates the circulating metabolome: evidence from a prospective cohort study, Eur. J. Prev. Cardiol. 29 (2022) 1448–1459. https://doi.org/10.1093/eurjpc/zwac060.

[35] K. Auro, A. Joensuu, K. Fischer, J. Kettunen, P. Salo, H. Mattsson, M. Niironen, J. Kaprio, J.G. Eriksson, T. Lehtimäki, O. Raitakari, A. Jula, A. Tiitinen, M. Jauhiainen, P. Soininen, A.J. Kangas, M. Kähönen, A.S. Havulinna, M. Ala-Korpela, V. Salomaa, A. Metspalu, M. Perola, A metabolic view on menopause and ageing, Nat. Commun. 5 (2014) 4708. https://doi.org/10.1038/ncomms5708.

[36] M. Maes, R. Verkerk, L. Delmeire, A. Van Gastel, F. Van Hunsel, S. Scharpé, Serotonergic markers and lowered plasma branched-chain-amino acid concentrations in fibromyalgia, Psychiatry Res. 97 (2000) 11–20. https://doi.org/10.1016/S0165-1781(00)00204-3.

[37] V. Ruggiero, M. Mura, E. Cacace, B. Era, M. Peri, G. Sanna, A. Fais, Free amino acids in fibromyalgia syndrome: relationship with clinical picture, Scand. J. Clin. Lab. Invest. 77 (2017) 93–97. https://doi.org/10.1080/00365513.2016.1269362.

[38] M. Clos-Garcia, N. Andrés-Marin, G. Fernández-Eulate, L. Abecia, J.L. Lavín, S. Van Liempd, D. Cabrera, F. Royo, A. Valero, N. Errazquin, M.C.G. Vega, L. Govillard, M.R. Tackett, G. Tejada, E. Gónzalez, J. Anguita, L. Bujanda, A.M.C. Orcasitas, A.M. Aransay, O. Maíz, A. López De Munain, J.M. Falcón-Pérez, Gut microbiome and serum metabolome analyses identify molecular biomarkers and altered glutamate metabolism in fibromyalgia, EBioMedicine 46 (2019) 499–511. https://doi.org/10.1016/j.ebiom.2019.07.031.

[39] A. Rus, J.A. López-Sánchez, J.M. Martínez-Martos, M.J. Ramírez-Expósito, F. Molina, M. Correa-Rodríguez, M.E. Aguilar-Ferrándiz, Predictive Ability of Serum Amino Acid Levels to Differentiate Fibromyalgia Patients from Healthy Subjects, Mol. Diagn. Ther. 28 (2024) 113–128. https://doi.org/10.1007/s40291-023-00677-8.

[40] I.J. Russell, J.E. Michalek, G.A. Vipraio, E.M. Fletcher, K. Wall, Serum amino acids in fibrositis/fibromyalgia syndrome, J. Rheumatol. Suppl. 19 (1989) 158–163.

[41] V. Menzies, A. Starkweather, Y. Yao, L.R. Thacker, T.J. Garrett, T. Swift-Scanlan, D.L. Kelly, P. Patel, D.E. Lyon, Metabolomic differentials in women with and without fibromyalgia, Clin. Transl. Sci. 13 (2020) 67–77. https://doi.org/10.1111/cts.12679.

[42] T. Blutstein, P.J. Baab, H.R. Zielke, J.A. Mong, Hormonal modulation of amino acid neurotransmitter metabolism in the arcuate nucleus of the adult female rat: a novel action of estradiol, Endocrinology 150 (2009) 3237–3244. https://doi.org/10.1210/en.2008-1701.

[43] H.K. Bhat, J.V. Vadgama, Role of estrogen receptor in the regulation of estrogen induced amino acid transport of System A in breast cancer and other receptor positive tumor cells, Int. J. Mol. Med. 9 (2002) 271–279.

[44] S. Della Torre, Beyond the x factor: relevance of sex hormones in NAFLD pathophysiology, Cells 10 (2021) 2502. https://doi.org/10.3390/cells10092502.

Disclosure section

-Author Contributions Statement:

Alma Rus contributed to conception and design of the study, analysis and interpretation of data, and drafted the article. Bárbara Coca-Guzmán contributed to analysis and interpretation of data and revised the article critically. Francisco Molina, María Correa-Rodríguez, José Manuel Martínez-Martos and María Jesús Ramírez-Expósito contributed to acquisition of data and revised the article critically. María Encarnación Aguilar-Ferrándiz contributed to conception and design of the study and revised the article critically. All authors saw and approved the final version, and no other person made a substantial contribution to the paper.

-Declaration of competing interest:

The authors declare that they have no competing interest.

-Funding source:

This work was supported by FEDER / Junta de Andalucía-Consejería de Transformación Económica, Industria, Conocimiento y Universidades (Spain) [grant number A-CTS-120-UGR20]. The funding source was not involved on study design, collection, analysis and interpretation of data, writing of the manuscript, or the decision to submit the article for publication.

-Availability of the research data:

The datasets generated during the current study are available from the corresponding author on reasonable request.

Table 1. Demographic and clinical data of women with fibromyalgia (FM) and healthy women.

	Group 1: Healthy Premenopausal Women (n=28)	Group 2: Healthy Postmenopausal Women (n=46)	Group 3: Premenopausal Women With FM (n=16)	Group 4: Postmenopausal Women With FM (n=52)	p-value
Age (years)	46.04±6.74	60.70±8.93	46.19±5.20	58.12±5.37	**0.000[a];0.000[b]**;0.939[c];0.092[d]
BMI (kg/cm²)	25.12±3.53	26.61±4.81	26.45±5.31	28.72±5.32	0.161[a];0.140[b];0.328[c];0.087[d]
FIQ-R	-	-	67.13±19.78	71.88±14.62	-;0.302[b];-;-
VAS	19.63±28.81	15.22±18.82	70.00±18.61	75.58±15.77	0.971[a];0.296[b]**;0.000[c];0.000[d]**
MFI	42.67±14.08	46.43±16.34	78.13±9.17	79.44±10.02	0.321[a];0.641[b]**;0.000[c];0.000[d]**
BAI	7.89±7.16	12.22±11.34	34.00±9.72	32.00±9.34	0.106[a];0.311[b]**;0.000[c];0.000[d]**
PSQI	5.44±2.88	7.30±4.40	15.38±4.41	15.33±3.51	0.098[a];0.711[b]**;0.000[c];0.000[d]**

Note. Data were expressed as mean ± standard deviation (SD). BMI: body mass index; FIQ-R: Revised Fibromyalgia Impact Questionnaire; VAS: Visual Analogic Scale; MFI: Multidimensional Fatigue Inventory: BAI: Beck Anxiety Inventory; PSQI: Pittsburgh Sleep Quality Index. [a]Comparisons between groups 1 and 2, [b]Comparisons between groups 3 and 4, [c]Comparisons between groups 1 and 3, [d]Comparisons between groups 2 and 4.

p-values < 0.05 are shown in bold.

Table 2. Serum concentrations of free amino acids in women with fibromyalgia (FM) and healthy women.

	Group 1: Healthy Premenopausal Women (n=28)	Group 2: Healthy Postmenopausal Women (n=46)	Group 3: Premenopausal Women With FM (n=16)	Group 4: Postmenopausal Women With FM (n=52)	p-value
Citrulline (Cit)	24.60±4.95	25.19±5.22	23.23±12.55	27.12±11.53	0.630[a];0.252[b];0.681[c];0.283[d]
Taurine (Tau)	59.70±13.31	61.89±13.25	89.56±33.72	81.35±32.36	0.495[a];0.383[b];**0.003[c];0.000[d]**
Carnosine (Car)	174.57±33.14	165.54±36.20	195.81±110.02	172.77±100.69	0.287[a];0.436[b];0.462[c];0.630[d]
γ-aminobutyric acid (GABA)	214.07±40.18	211.52±40.22	198.25±55.59	221.46±66.33	0.792[a];0.209[b];0.282[c];0.366[d]
Aspartic Acid (Asp)	21.88±3.46	21.95±3.08	29.03±9.67	35.08±16.48	0.824[a];0.305[b];**0.005[c];0.000[d]**
Glutamic acid (Glu)	37.16±3.41	37.98±3.33	82.31±37.83	71.17±31.83	0.300[a];0.394[b];**0.000[c];0.000[d]**
Aminoadipic acid (Aad)	0.59±0.34	0.69±0.34	0.96±1.13	1.30±0.99	0.233[a];0.078[b];0.751[c];**0.007[d]**
Asparragine (Asn)	39.25±2.56	39.25±2.38	53.84±29.42	52.11±21.81	0.929[a];0.908[b];0.367[c];**0.001[d]**
Serine (Ser)	118.19±7.42	117.94±8.63	128.63±61.46	137.68±64.88	0.907[a];0.707[b];0.836[c];0.552[d]
Glutamine (Gln)	134.57±5.36	132.15±5.58	158.27±55.84	151.62±42.92	0.079[a];0.931[b];0.495[c];0.101[d]
Histidine (His)	28.85±1.07	28.66±0.90	51.51±26.39	45.74.22.42	0.406[a];0.603[b];**0.004[c];0.000[d]**
Glycine (Gly)	52.54±13.48	52.39±12.44	71.16±30.83	83.51±37.59	0.938[a];0.193[b];**0.025[c];0.000[d]**
Threonine (Thr)	93.06±5.08	92.16±4.76	169.93±121.60	151.19±83.49	0.442[a];0.942[b];0.188c;**0.000[d]**
Arginine (Arg)	61.80±5.12	60.99±5.57	65.80±24.54	73.64±24.52	0.544[a];0.183[b];0.696[c];**0.023[d]**
5-methyl-histidine (5-met-his)	2.38±1.01	2.45±0.94	3.59±3.19	3.87±2.77	0.688[a];0.588[b];0.608[c];**0.031[d]**
Alanine (Ala)	324.08±17.42	320.72±17.89	231.90±76.16	233.75±95.92	0.504[a];0.851[b];**0.000[c];0.000[d]**
Tyrosine (Tyr)	55.92±7.59	57.14±8.80	74.79±35.79	70.44±33.51	0.465[a];0.778[b];0.252[c];0.100[d]
3-methyl-histidine (3-met-his)	80.84±12.87	84.20±13.02	160.48±117.15	136.24±102.61	0.277[a];0.444[b];0.054[c];0.093[d]
Valine (Val)	188.86±5.46	187.46±5.67	271.69±144.60	284.75±169.39	0.259[a];0.919[b];0.261[c];**0.022[d]**
Methionine (Met)	20.79±1.87	20.67±2.15	30.19±16.79	30.29±16.96	0.778[a];0.948[b];0.148[c];**0.041[d]**
Isoleucine (Ile)	49.35±7.84	46.86±8.78	77.85±40.49	69.13±37.23	0.233[a];0.525[b];0.130[c];**0.003[d]**
Phenylalanine (Phe)	55.80±6.71	54.68±7.28	67.11±23.44	80.94±32.33	0.422[a];0.152[b];0.196[c];**0.000[d]**
Tryptophan (Trp)	67.23±13.31	73.66±14.32	99.77±80.29	101.01±74.13	0.047[a];0.817[b];0.981[c];0.165[d]
Leucine (Leu)	117.91±24.10	114.85±24.61	181.35±86.95	183.60±97.31	0.525[a];0.885[b];**0.013[c];0.001[d]**
Ornithine (Orn)	364.58±71.20	369.14±65.72	418.09±260.67	552.64±305.13	0.806[a];0.062[b];0.661[c];**0.001[d]**
Lysine (Lys)	114.42±5.78	113.77±6.83	129.43±82.60	158.46±87.63	0.798[a];0.214[b];0.479[c];0.057[d]

Note. Data are expressed as mean ± standard deviation (SD). Concentrations were expressed as picomoles of amino acid per microliter (pmoles/µL). [a]Comparisons between groups 1 and 2, [b]Comparisons between groups 3 and 4, [c]Comparisons between groups 1 and 3, [d]Comparisons between groups 2 and 4.
p-values < 0.05 are shown in bold.

Table 3. Groupings and ratios of amino acids in women with fibromyalgia (FM) and healthy women.

	Group 1: Healthy Premenopausal Women (n=28)	Group 2: Healthy Postmenopausal Women (n=46)	Group 3: Premenopausal Women With FM (n=16)	Group 4: Postmenopausal Women With FM (n=52)	p-value
BCAAs	356.19±29.49	349.19±28.95	530.84±238.34	537.52±253.65	0.260[a];0.988[b];0.060[c];**0.000[d]**
LNAAs	467.92±33.80	461.01±32.04	672.75±268.76	688.91±291.22	0.413[a];0.862[b];0.060[c];**0.000[d]**
EAAs	669.15±28.66	659.14±30.77	978.99±417.27	1003.86±422.82	0.111[a];0.862[b];0.107[c];**0.000[d]**
NEAAs	1238.39±80.88	1239.63±77.39	1351.86±346.26	1499.71±428.17	0.991[a];0.198[b];0.407[c];**0.000[d]**
BAAs	569.66±73.73	572.57±66.32	664.84±336.33	830.22±374.19	0.876[a];0.102[b];0.643[c];**0.000[d]**
Arg/Orn ratio	0.18±0.04	0.17±0.03	0.19±0.08	0.19±0.16	0.642[a];0.232[b];0.980[c];**0.046[d]**
TSM ratio	2.46±0.64	2.59±0.68	3.62±2.89	3.16±2.80	0.448[a];0.468[b];0.608[c];0.655[d]
GABR ratio	0.16±0.03	0.16±0.03	0.18±0.07	0.17±0.14	0.735[a];0.227[b];0.863[c];0.077[d]

Note. Data are expressed as mean ± standard deviation (SD). Concentrations of BCAAs, LNAAs, EAAs, NEAAs, and BAAs were expressed as picomoles per microliter (pmoles/μL). BCAAs: Branched-chain amino acids; LNAAs: Large neutral amino acids; EAAs: Essential amino acids; NEAAs: Non-essential amino acids; BAAs: Basic amino acids; Arg: Arginine; Orn: ornithine; GABR ratio: global arginine bioavailability ratio. [a]Comparisons between groups 1 and 2, [b]Comparisons between groups 3 and 4, [c]Comparisons between groups 1 and 3, [d]Comparisons between groups 2 and 4.
p-values < 0.05 are shown in bold.

Declaration of competing interest: The authors declare that they have no competing interest.

Ethical Statement: The present study was carried out in accordance with the Declaration of Helsinki of the World Medical Association (WMA). The study was approved by the Granada Provincial Research Ethics Committee (Junta de Andalucía, Spain), with approval number 1797-N-17. All participants provided written informed consent and did not receive financial incentive.