UNIVERSIDAD DE EXTREMADURA

TESIS DOCTORAL

INFLUENCIA DE LA COMORBILIDAD DEL SÍNDROME DE FATIGA CRÓNICA EN PACIENTES CON FIBROMIALGIA SOBRE LA ACTIVIDAD FÍSICA, LA CALIDAD DE VIDA Y LA RESPUESTA PSICOINMUNONEUROENDOCRINA

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PROGRAMA DE DOCTORADO EN BIOMARCADORES DE SALUD Y ESTADOS PATOLÓGICOS

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AÑO 2023

Departamento de Fisiología (Facultad de Ciencias)

Instituto Universitario de Investigación Biosanitaria de Extremadura (INUBE)

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CERTIFICAN:

Que la Tesis Doctoral presentada a superior juicio del tribunal, que designe la Universidad de Extremadura, por **D. Eduardo Otero Calderón**, titulada *"Influencia de la comorbilidad del síndrome de fatiga crónica en pacientes con fibromialgia sobre la actividad física, la calidad de vida y la respuesta psicoinmunoneuroendocrina"*, ha sido realizada bajo nuestra dirección en el Programa de Doctorado **"Biomarcadores de Salud y Estados Patológicos"** (R-012) de la Escuela Internacional de Doctorado de la Universidad de Extremadura, reúne, a nuestro parecer, todos los requisitos para ser defendida públicamente; por lo que otorgamos nuestra conformidad para ello.

En Badajoz a 8 de junio de 2023

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Esta Tesis Doctoral ha sido financiada con Ayudas a Grupos de Investigación (GR18009; GR21079) concedidas al Grupo de Investigación "Inmunofisiología: Estrés, Ejercicio, Envejecimiento y Salud" (CTS007) por la Junta de Extremadura-Fondo Europeo de Desarrollo Regional; Ayudas para el fomento de la contratación de personal de apoyo a la investigación en la Comunidad Autónoma de Extremadura (TE-0027-19); y un contrato de colaboración entre la Universidad de Extremadura y LABORATORIOS HEEL ESPAÑA S.A.U. para la realización del proyecto de investigación "Evaluación inmunofisiológica del simbiótico Gasteel Plus en la fibromialgia" (Ref. 056/23).

Agradecimientos

Me gustaría expresar mi más sincero agradecimiento a todas aquellas personas que han contribuido directa o indirectamente durante estos tres años de tesis doctoral:

En primer lugar, al Dr. Eduardo Ortega Rincón, director de esta tesis doctoral. Hace tres años me era imposible imaginar el poder llegar a realizar una Tesis Doctoral, y tú me has dado esa oportunidad. Gracias por confiar en mí, incluso cuando yo no lo hacía. Me siento muy afortunado de haber podido aprender de tí, tanto en la ciencia como en cualquier aspecto de la vida. Gracias por enseñarme que no hace falta ser tan "de proteínas", sino confiar en tí mismo y aplicar el sentido común, y que, ningún jugador es tan bueno como todos juntos. Gracias por contagiarme tu pasión por la ciencia y tu dedicación. Gracias, amigo, por estar ahí siempre.

A la Dra. María Dolores Hinchado Sánchez-Moro, codirectora de esta tesis doctoral, por apostar y dar la cara siempre por mí, por ser mi amiga, por enseñarme a darle importancia a lo verdaderamente importante, por todos esos momentos, más buenos que malos y porque siempre quedará una cerveza más. Espero poder devolverte algún día todo lo que has compartido conmigo, y no hablo de imperdibles o de chicles, sino de tu constancia, tu trabajo, y el cariño y la dedicación que le pones a todo lo que haces. Gracias, por ser crucial en estos 3 años, y espero que en muchos más.

A mis compañeras del grupo de "Inmunofisiología: Estrés, Ejercicio, Envejecimiento y Salud" del INUBE, especialmente a la Dra. Leticia Martín-Cordero, la Dra. Isabel Gálvez Galán y mi compañera María del Carmen Navarro Pérez, por vuestro apoyo incondicional y por tantos buenos momentos en el laboratorio. Me siento muy afortunado de pertenecer a este grupo de investigación y, por supuesto, de amigos. También a la Dra. Silvia Torres Piles y a la Dra. Julia Marín Expósito, con las que compartí algunos experimentos en el grupo de investigación.

A la Dra. Carmen Daniela Quero Calero por su contribución a esta tesis doctoral, su ayuda desinteresada en todo momento y por todos esos buenos momentos que hemos pasado.

A las asociaciones de fibromialgia de Extremadura: AFIBROEX (Cáceres), AFIBRODON (Don Benito) y EXISTIMOS (Badajoz) y todas sus pacientes, por su participación y su completa disposición. En especial, me gustaría agradecer a Isabel Gaitán y a Chari Pírez por su gran ayuda y su interés por este proyecto de tesis.

A todas aquellas voluntarias "sanas" que han participado y que han hecho factible la realización de este proyecto de tesis. Sin vosotras no hubiera sido posible.

Al Servicio de Prevención de la Universidad de Extremadura, en especial a Antonio Pérez, por su colaboración en las valoraciones de composición corporal necesarias para esta tesis doctoral.

A Manu y a Gloria, aparte de vuestro apoyo incondicional, por contribuir con vuestro tiempo en labores de enfermería.

A mi familia: mis padrinos, Miguel y Oli, mis tíos: José Luis, Juan Carlos, Manoli y Bego, y a Elvi, por toda su implicación y su participación. Gracias de corazón. A mis padres y mi hermano, por permitirme hacer lo que me gusta y regalarme vuestra vida, estando siempre a mi lado y apoyándome en los momentos más difíciles. Me siento muy afortunado de ser vuestro hijo y hermano.

A todos mis amigos por acompañarme en este camino, en especial a Ale, Jau, Juande, Julio y Sonia, gracias por estar en todo momento, tanto los buenos como los malos y a mi primo Javi, por ser un pilar fundamental y estar siempre al pie del cañón.

A Elen, por aguantarme y apoyarme en todo lo que hago. Han sido tres años increíbles y tengo mucha suerte de haber podido compartirlos contigo. Gracias por creer en mí y por estar siempre, tanto en lo bueno como en lo malo. Nunca podré agradecerte lo suficiente.

Por último, a mis abuelos, por toda la fuerza que me habéis mandado, desde donde quiera que sea.

“Eppur si muove”

Galileo Galilei

Índice de contenidos

Introducción.

A. Hipótesis y objetivos

La presente tesis doctoral se realiza por compendio de tres publicaciones enfocadas en el síndrome de fibromialgia (FM), y la influencia que, sobre los aspectos de realización de actividad física, calidad de vida y estado psicoinmunoneuroendocrino evaluados presenta un diagnóstico previo con el síndrome de fatiga crónica (SFC). Estos dos síndromes, cuyas fisiopatologías siguen sin esclarecerse, son comúnmente codiagnosticados, en ocasiones pudiendo provocar un sobrediagnóstico de alguno de ellos, dadas sus semejanzas sintomatológicas. A pesar de su prevalencia creciente sobre todo en mujeres y especialmente en los países desarrollados, su etiopatogenia sigue sin estar clara debido a la ausencia de biomarcadores específicos relativos al dolor, la fatiga y la calidad de vida, lo que conduce a que su diagnóstico diferencial siga siendo un reto, razón por la cual estas enfermedades sean comórbidas entre sí en más de un 40% de pacientes. Sin embargo, existe poca literatura que tenga en cuenta la comorbilidad de ambos síndromes.

Basándonos en la **hipótesis** de partida de que la comorbilidad del SFC en las pacientes con FM pudiera deteriorar aún más su capacidad para realizar actividad física, sus niveles de estrés y, en definitiva, el dolor y la fatiga que dificultan las actividades cotidianas y, por tanto, la calidad de vida; correlacionándose con un aumento del desequilibrio en la respuesta psicoinmunoneuroendocrina, planteamos el siguiente **objetivo general:** evaluar diferencias en la respuesta psicoinmunoneuroendocrina y en la calidad de vida asociada al dolor, la fatiga y el estrés, fundamentalmente en relación a los niveles de actividad física y sedentarismo, en mujeres diagnosticadas con síndrome de fibromialgia (FM), con o sin un codiagnóstico previo de síndrome de fatiga crónica (SFC), utilizando como referencia un grupo de mujeres "sanas" del mismo rango de edad sin FM, SFC o cualquier otra patología inflamatoria o reumática. Para desarrollar nuestro objetivo general, lo dividimos en los **subobjetivos** que se enumeran a continuación:

1. Evaluar cómo afecta el codiagnóstico de SFC y la realización de ejercicio físico habitual (EFH) a la salud psicológica y a la calidad de vida percibidas en pacientes con fibromialgia, a través de cuestionarios científicamente validados relativos al dolor, la fatiga, el estrés, la ansiedad, la depresión y la ansiedad frente a la COVID-19.

2. Profundizar en los trastornos psicoinmunoneuroendocrinos de las pacientes con FM y comprobar si un previo diagnóstico de SFC crónica pudiera afectarlos; todo ello determinado tanto de forma percibida, a través de cuestionarios validados científicamente, como de forma objetiva, a través de acelerometría y de biomarcadores inmunofisiológicos sistémicos de inflamación y de ansiedad-estrés.

3. Analizar en pacientes con FM, con o sin SFC codiagnosticada, los posibles efectos de un simbiótico comercial sobre los parámetros de calidad de vida, inflamación y estrés, tanto de forma percibida como objetiva mencionados en los dos objetivos anteriores.

B. Presentación y justificación de las publicaciones

Aunque se ha demostrado que existe una relación positiva entre el EFH y la calidad de vida percibida en pacientes con FM, existe poca literatura que tenga en cuenta cómo podría afectar el codiagnóstico con SFC. Por tanto, en primer lugar, en esta investigación quisimos conocer si el codiagnóstico de SFC influiría tanto en la realización de EFH, como en la calidad de vida asociada al dolor, en el estrés, la fatiga, la ansiedad, la depresión y la ansiedad frente a la COVID-19, en pacientes con FM de forma percibida o subjetiva **(Publicación 1)**. Así, en una muestra de mujeres diagnosticadas de FM, con o sin codiagnóstico previo de SFC, encontramos que, paradójicamente, el codiagnóstico de SFC no influyó en el porcentaje de pacientes que realizaban EFH, ni en el ya deteriorado estado psicológico que presentaron las pacientes de FM, frente a un grupo de referencia del mismo rango de edad, todo ello evaluado a través de cuestionarios validados científicamente. A su vez, pudimos comprobar como las pacientes que reportaron realizar EFH manifestaron menos niveles de ansiedad y miedo frente a la COVID-19, con respecto al grupo de referencia. Con ello hemos podido materializar el primer objetivo de esta tesis.

Con estos resultados previos, y basándonos en la necesidad de encontrar biomarcadores objetivos que reflejen los síntomas y la salud percibida de las pacientes con FM y que puedan ayudar a realizar un diagnóstico diferencial entre los dos síndromes, profundizamos en el estado psicoinmunoneuroendocrino de estas pacientes. Todo ello se llevó a cabo relacionando los efectos percibidos con los biomarcadores objetivos de actividad física, estrés/ansiedad y respuesta inflamatoria, teniendo en cuenta además la influencia del codiagnóstico previo de SFC; desarrollando así nuestro segundo objetivo **(Publicación 2)**. Por tanto, en grupos representativos de menor tamaño muestral, extraídos de cada uno de los grupos definidos en la publicación anterior, encontramos que el deterioro de la salud percibida en pacientes con FM se corroboró tanto por el deterioro de los parámetros objetivos de actividad física medidos mediante acelerometría, como a través de biomarcadores de inflamación y estrés. Además, mientras que la salud percibida relacionada con la fatiga y la capacidad de realizar actividad física, los trastornos psicológicos y el dolor no se veían afectados por un diagnóstico previo de SFC; los biomarcadores inmunoneuroendocrinos objetivos relacionados con el estrés, la depresión y el dolor sólo se manifestaban significativamente elevados en pacientes sin diagnóstico de SFC. Esto sugiere un posible sobrediagnóstico de la FM en pacientes con SFC cuando se evalúa sólo a través de los síntomas percibidos y no con parámetros inmunofisiológicos objetivos.

Por último, con el propósito de ayudar a una correcta preinscripción de ayudas terapéuticas no farmacológicas, que mejoren la calidad de vida de pacientes con FM a través de una mejor regulación inmunofisiológica, realizamos una intervención con un simbiótico (prebióticos y probióticos) comercial de libre consumo, Todo ello, en el contexto de la importancia del eje “intestino-cerebro”, continuando conjuntamente con el mismo objetivo de conocer la influencia de un codiagnóstico previo de SFC **(Publicación 3)**. Con ello, completábamos el tercer y último objetivo de la presente Tesis Doctoral. Encontramos que el simbiótico, además de producir mejoras significativas en los niveles percibidos de estrés,

ansiedad y depresión, generaba un efecto beneficioso sobre el desequilibrio inmunoneuroendocrino que presentaban las mujeres con FM, efecto que se produce fundamentalmente en pacientes sin diagnóstico previo de SFC.

Este estudio diferencial está justificado en el contexto de los problemas en cuanto a la capacidad disminuida de realización de actividad diaria que presentan estas pacientes como consecuencia del dolor y otras afecciones nerviosas. Entendemos que puede contribuir a validar y objetivar los aspectos más subjetivos y de calidad de vida de las pacientes. Con todo lo indicado se justifica plenamente la coherencia e importancia unitaria de la Tesis a través del compendio de estas tres publicaciones, cada una de ellas abordando y resolviendo cada uno de los objetivos parciales propuestos, que nos han llevado a establecer las conclusiones adecuadas acordes al objetivo general y la hipótesis planteada, que únicamente ha podido ser validada parcialmente como se explicará y concluirá más adelante. A ello, y durante el período del desarrollo de toda la tesis doctoral, también ha ido contribuyendo la presentación de los resultados obtenidos en los principales Congresos Nacionales e Internacionales de nuestro ámbito, como queda reflejado también en el anexo final.

Además, la presente investigación aúna en cada uno de sus tres objetivos tres aspectos básicos de la investigación biosanitaria en humanos: la salud percibida de un síndrome con etiología y fisiopatología sin esclarecer, la búsqueda de biomarcadores objetivos que ayuden a la prevención, diagnóstico y establecimiento de estrategias terapéuticas para mejorar la calidad de vida de las pacientes y la validación de ayudas terapéuticas (particularmente no farmacológicas) a través de la mejora de estos biomarcadores objetivos. Además, y también lo consideramos importante, se ha desarrollado tanto con financiación pública como con financiación privada, ésta en su último objetivo y que ha permitido la transferencia de investigación a la sociedad no sólo en el ámbito público sino tambien a través del sector privado.

C. Listado de aportaciones científicas que constituyen la tesis doctoral por compendio

- Publicaciones: artículos en revistas

4. Hinchado, M. D., **Otero, E***., Navarro, M. D. C., Martín-Cordero, L., Gálvez, I., y Ortega, E. (2022). Influence of Codiagnosis of Chronic Fatigue Syndrome and Habitual Physical Exercise on the Psychological Status and Quality of Life of Patients with Fibromyalgia. *Journal of Clinical Medicine,* 11(19), 5735. https://doi.org/10.3390/jcm11195735 **IF: 4.964, Q2. (Primer Tercil).**

5. Otero, E., Gálvez, I., Ortega, E.*, y Hinchado, M. D. (2023). Influence of Chronic Fatigue Syndrome Codiagnosis on the Relationship between Perceived and Objective Psychoneuro-Immunoendocrine Disorders in Women with Fibromyalgia. *Biomedicines,* 11(5), 1488. https://doi.org/10.3390/biomedicines11051488 **IF: 4.757, Q2 (Primer Tercil).**

6. Hinchado, M. D., Quero-Calero, C. D*., **Otero, E.**, Gálvez, I., y Ortega, E. (2023). Synbiotic Supplementation Improves Quality of Life and Inmunoneuroendocrine Response in Patients with Fibromyalgia: Influence of Codiagnosis with Chronic Fatigue Syndrome. *Nutrients,* 15(7), 1591. https://doi.org/10.3390/nu15071591 **IF: 6.706, Q1**

Publicación 1.

Hinchado, M. D., Otero, E*., Navarro, M. D. C., Martín-Cordero, L., Gálvez, I., y Ortega, E. (2022). Influence of Codiagnosis of Chronic Fatigue Syndrome and Habitual Physical Exercise on the Psychological Status and Quality of Life of Patients with Fibromyalgia. *Journal of Clinical Medicine,* 11(19), 5735. https://doi.org/10.3390/jcm11195735

Título: *"Influencia del Codiagnóstico del Síndrome de Fatiga Crónica y del Ejercicio Físico Habitual sobre el Estado Psicológico y la Calidad de Vida de Pacientes con Fibromialgia"*

Resumen

La fibromialgia (FM) y el síndrome de fatiga crónica (SFC) son dos enfermedades que se codiagnostican con frecuencia y presentan muchas similitudes, como la escasa tolerancia al ejercicio físico. Aunque el ejercicio se recomienda en su rutina diaria para mejorar la calidad de vida, se sabe poco sobre cómo afecta el codiagnóstico de SFC. Mediante cuestionarios validados científicamente, se evaluó el estado psicológico y la calidad de vida de pacientes con FM (n = 70) y cómo influye en esos aspectos el ejercicio físico habitual (EFH) reportado por pacientes con sólo FM (sólo FM, n = 38) o codiagnosticados con SFC (FM + SFC, n = 32). Se utilizó un grupo de referencia del mismo rango de edad de mujeres "sanas" sin FM (RG, n = 70). El grupo de sólo FM presentó un peor estado psicológico y calidad de vida en comparación con el GR, sin influencia del codiagnóstico de SFC. Las pacientes de los grupos de sólo FM y FM + SFC que realizan EFH presentaron mejores niveles de estrés y ansiedad estado, pero sin diferencias entre ellos. La depresión y la ansiedad rasgo mejoraron sólo en las mujeres con sólo FM. El codiagnóstico de SFC no empeora el deterioro psicológico y de la calidad de vida de las pacientes con FM y no tiene una gran influencia en el efecto positivo del EFH.

Journal of
Clinical Medicine

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Article

Influence of Codiagnosis of Chronic Fatigue Syndrome and Habitual Physical Exercise on the Psychological Status and Quality of Life of Patients with Fibromyalgia

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Abstract: Fibromyalgia (FM) and Chronic Fatigue Syndrome (CFS) are two diseases that are frequently codiagnosed and present many similarities, such as poor tolerance to physical exercise. Although exercise is recommended in their daily routine to improve quality of life, little is known about how CFS codiagnosis affects that. Using scientifically validated questionnaires, we evaluated the psychological state and quality of life of patients with FM (n = 70) and how habitual physical exercise (HPE) reported by patients with only FM (FM-only n = 38) or codiagnosed with CFS (FM + CFS, n = 32) influences those aspects. An age-matched reference group of "healthy" women without FM (RG, n = 70) was used. The FM-only group presented a worse psychological state and quality of life compared to RG, with no influence of CFS codiagnosis. The patients of the FM-only and FM + CFS groups who perform HPE presented better levels of stress and state anxiety, but with no differences between them. Depression and trait anxiety improved only in women with just FM. CFS codiagnosis does not worsen the psychological and quality of life impairment of FM patients and does not have a great influence on the positive effect of HPE.

Keywords: fibromyalgia; chronic fatigue syndrome; exercise; stress; COVID-19

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Citation: Hinchado, M.D.; Otero, E.; Navarro, M.d.C.; Martín-Cordero, L.; Gálvez, I.; Ortega, E. Influence of Codiagnosis of Chronic Fatigue Syndrome and Habitual Physical Exercise on the Psychological Status and Quality of Life of Patients with Fibromyalgia. *J. Clin. Med.* **2022**, *11*, 5735. https://doi.org/10.3390/jcm11195735

Academic Editors: Indre Bileviciute-Ljungar and Antonio Naranjo Hernandez

Received: 22 June 2022
Accepted: 24 September 2022
Published: 28 September 2022

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1. Introduction

Approximately 6.3% of the world population suffers from fibromyalgia (FM), being more frequent in women, according to the World Fibromyalgia Association, and although it is a chronic disease with no fully established etiology, moderate physical exercise is the main proven, validated non-pharmacological therapy for the management of FM according to the EULAR [1]. The problem lies in the fact that the pain and fatigue felt by people with FM often hamper participation in regular daily activities and even more so, in sporting activities. Despite that, physical exercise is the most recommended adjuvant in the treatment for the improvement of the predominant symptom: pain [2]. In addition, people with FM who have a better physical shape also have a reduced manifestation of this condition [3]. Although a positive relationship between habitual physical exercise (HPE) and quality of life in FM has been clearly described [4–8], one of the great challenges of FM is the frequent association of this syndrome with different pathologies, such as Chronic Fatigue Syndrome (CFS). CFS is characterized by disabling fatigue lasting more than 3 months, which further worsens the course and development of the disease and

makes it even more difficult to acquire routines that include exercise. For people with CFS, exercise also seems to significantly improve health status, physical fitness, and quality of life, thus having a direct impact in fatigue, but literature is still scarce in this regard. People with CFS tend to have an aversion to exercise to avoid symptom aggravation. Because of this, they do not (attempt to) engage in physical activities and become completely sedentary [9]. Therefore, little is known about the benefits of daily exercise practice when these two syndromes are comorbid, which occurs in 20–81% of FM patients [10,11]. In fact, several authors already pointed out that it is important to consider the comorbidity of both pathologies when recommending physical exercise, since they found differences in the perception of the intensity of effort and a decrease in systolic blood pressure [12]. Moreover, FM+CFS patients also had moderately lower total peripheral vascular resistance and experienced greater musculoskeletal pain during exercise than FM-only patients. In women with only FM, aerobic exercise improves the levels of anxious and depressive symptoms that develop in numerous patients with this pathology [6]. Benefits of exercise therapy have also been found in patients diagnosed with only CFS, especially in terms of fatigue, without evidence that it may worsen the course of the disease. However, more studies are needed to examine the influence of CFS comorbidity on the effects of HPE in FM patients, particularly concerning quality of life, anxiety, and depression [13]. Therefore, in this research, it seems plausible to question how the codiagnosis of CFS has a perceived influence on the effects of reported regular physical exercise on psychological well-being and quality of life in patients with FM.

Previous studies in our laboratory have shown that pain, fatigue, and decreased quality of life of FM patients are due, at least in part, to a neuroimmunoendocrine dysregulation in stress and inflammation biomarkers. This dysregulation is improved with the performance of HPE [5,7,8,14]. Nevertheless, and taking into account that fatigue is the main symptom in CFS patients, it is plausible to hypothesize that CFS codiagnosis in FM patients could affect their quality of life and psychological state, as well as the effect of habitual physical activity on these aspects. In this context, and before delving into neuroimmunoendocrine biomarkers, the objective of this study was to ascertain how codiagnosis of CFS and the performance of HPE affects psychological health and perceived quality of life in patients with FM, all of this in an easy and non-invasive way, which is part of a broader investigation. This will allow a deeper insight into the influence of HPE (the main non-pharmacological therapy available in this pathology) reported by the patients, on psychological aspects and quality of life in patients with FM. Understanding of these aspects will contribute to clarifying other psycho-neuroimmunoendocrine axis mechanisms and explore strategies that improve the course of both diseases.

2. Materials and Methods

2.1. Participants

Extremadura is an autonomous community of Spain with approximately 1,000,000 inhabitants, with a very homogeneous population in terms of lifestyle. The majority of the population is assisted by the Spanish National Health System, in which patient associations play a relevant role. Extremadura is also a reference region in Spain in health research [15,16], and particularly in patients with fibromyalgia and in the effects of exercise internationally.

The study was carried out in 70 women diagnosed with FM (Total FM group), all of them aged between 40 and 65 years, and belonging to the FM associations of Extremadura. A total of 38 of these women had a diagnosis of only FM (FM-only group), and the remaining 32 also had a previous codiagnosis of CFS (FM+ CFS group). In total, 70 women of the same age range were used as a reference group of "healthy" women, not diagnosed with FM, CFS, or any other inflammatory or rheumatic pathology (RG), as well as pathologies affecting depression, anxiety, and/or pain. Figure 1 shows the flow charts of participants in the study.

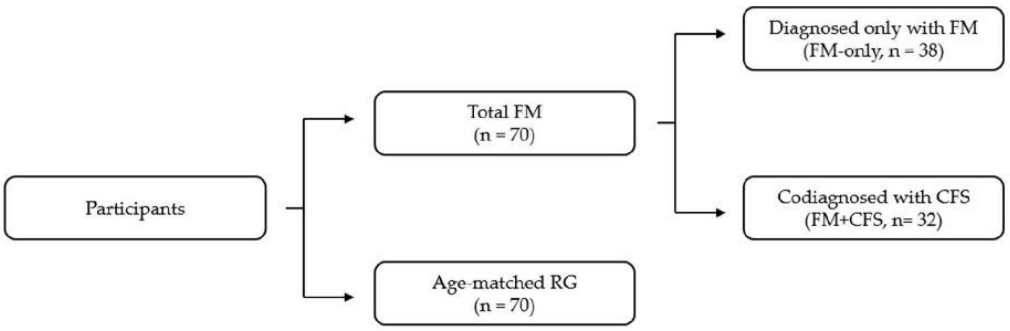

Figure 1. Flow charts of participants in the study. FM = Fibromyalgia, RG = Reference Group, CFS = Chronic Fatigue Syndrome.

It is important to highlight, as a strength of this study, that among the volunteers belonging to the FM associations of Extremadura, all of those who met the inclusion criteria were selected: (a) diagnosis of CFS and/or FM by rheumatologists or internal medicine professionals according to ACR diagnostic criteria for FM patients [17], and Fukuda and co-workers criteria for CFS patients [18], (b) aged between 40 and 65 years, (c) not having a diagnosis of depression, and (d) not suffering from multiple chemical sensitivity. Table 1 shows the main characteristics of that participants: anthropometrics data, employment status, and common comorbidities. All participants were Caucasian women and had been diagnosed with FM (with or without a previous diagnosis of CFS) for more than two years. There were no significant differences in age and BMI between the groups. With respect to professional work activity and the most common comorbidities of participants, no differences were found between FM-only and FM + CFS groups, but they presented significant differences with respect to the RG group, particularly in white collar workers and retired people as well as women diagnosed with osteoarthritis. Medication history was very diverse in each patient, but all of them were prescribed, at time of the present study, with different types of anti-inflammatory and analgesic drugs (e.g., ibuprofen, dexketoprofen, paracetamol, tramadol). All participants of experimental and control groups diagnosed with hypothyroidism were treated with levothyroxine.

Table 1. Anthropometric characteristics, employment status, and the most common comorbidities of the participants.

	RG (N = 70)	FM-Only (N = 38)	FM + CFS (N = 32)	Statistical Significance
Gender (%)	Women (100%)	Women (100%)	Women (100%)	
Ethnic group (%)	Caucasian (100%)	Caucasian (100%)	Caucasian (100%)	
Age (years)	53.93 ± 8.27	54.82 ± 8.52	57.31 ± 7.86	$p > 0.05$
BMI (kg/m^2)	25.72 ± 4.75	26.42 ± 5.25	26.82 ± 5.16	$p > 0.05$
Duration of FM or CFS diagnosed (years)	___	>2	>2	
Employment status:				**Chi-Square (X^2)** $p < 0.001$ ($X^2 < 0.001$)
- **Blue collar workers (%)**	8.6	15.8	12.5	
- **White collar workers (%)**	77.1	28.9 *	18.8 *	
- **Unemployed (%)**	8.6	26.3 *	18.8	
- **Medical leave (%)**	___	5.3	18.6 *	
- **Retired (%)**	5.7	23.7 *	31.3 *	
Common comorbidities				**Chi-Square (X^2)**
- **Hypothyroidism (%)**	11.4	28.9	37.5 *	$p < 0.01$ ($X^2 = 0.007$)
- **Hypertension (%)**	8.6	13.2	18.8	$p > 0.05$ ($X^2 = 0.332$)
- **Osteoarthritis (%)**	___	28.9 *	18.8 *	$p < 0.001$ ($X^2 < 0.001$)

Data are expressed as mean ± SEM and as percentage (%). RG: Reference Group, FM: Fibromyalgia, CFS: Chronic Fatigue Syndrome, BMI: Body Mass Index. * $p < 0.05$, with respect to reference group (post hoc Z-Test).

Written informed consent was also requested from all participants before participating in the study. The research had been previously approved by the Bioethics Committee of the University of Extremadura by the Directives of the Council of Europe and the Declaration of Helsinki (registration number 13/2020). This study was registered with ClinicalTrials.gov (identifier: NCT05323838: available on the website).

2.2. Instruments

To evaluate the perceived quality of life in our experimental groups, we used the following scientifically validated questionnaires.

The Beck Depression Inventory (BDI) was used to determine the presence of signs of depression during the last week, including the day of the test. Higher scores are related to greater signs of depression. According to the final score, perceived depression can be classified as: mild (between 10–19), moderate (between 20–30) or severe (>30) [19]. Spanish version of Sanz et al. [20] was used.

The Perceived Stress Scale (PSS) is a self-report instrument that evaluates the level of perceived stress during the last month, consisting of 14 items with a five-point Likert scale response format (0 = never, 1 = almost never, 2 = sometimes, 3 = fairly often, 4 = very often). The total score of the PSS is obtained by reversing the scores of items 4, 5, 6, 7, 9, 10 and 13 (as follows: 0 = 4, 1 = 3, 2 = 2, 3 = 1 and 4 = 0) and then adding up the scores of the 14 items. A higher total score corresponds to a higher level of perceived stress [21]. The Spanish version of Remor [22] was used.

The State-Trait Anxiety Inventory (STAI) is a 40-item self-report questionnaire designed to assess two independent concepts of anxiety: state anxiety (transient emotional condition) and trait anxiety (relatively stable characteristic of anxiety propensity). Each subscale comprises a total of 20 items in a four-point Likert response system according to intensity (0 = not at all/almost never, 1 = somewhat/sometimes, 2 = moderately so/often, 3 = very much so/almost always). The total score on each of the subscales ranges from 0 to 60 points [23]. Higher scores indicate a higher state of anxiety. Spanish version by Buela-Casal & Guillén-Riquelme [24] was used in the present study.

The Brief Pain Inventory (BPI) is a self-administered questionnaire that was originally designed to assess cancer pain [25]. It is now also used as a generic pain questionnaire for other chronic pain conditions [26]. It consists of two basic magnitudes: intensity, and interference of pain with the patient's activities of daily living, both scored on scales from 0 "no pain" to 10 "the worst pain". Higher scores are directly correlated to a higher perception of pain. The Spanish version of the BPI has proven to be valid for measuring the intensity of pain and its impact on activities of daily living under routine clinical practice conditions [27].

The Brief Fatigue Inventory (BFI) is a questionnaire that assesses the level of fatigue and its impact on the activities of daily living of the subjects in the last 24 h [28]. The first three items evaluate the subject according to their perceived fatigue on a scale from 0 "no fatigue" to 10 "the worst fatigue", and the remaining items evaluate the interference of fatigue in different aspects of the subjects' lives (general activity, mood, walking ability, work, relations with other people, and enjoyment of life) on a scale of 0 "does not interfere" to 10 "completely interferes". The higher the score of both items, the greater the perceived fatigue. The Spanish version of Valenzuela et al. [29] was used.

The main objective of the Healthy Lifestyle and Personal Control Questionnaire (HLPCQ) is to detect and quantify lifestyle patterns that reflect the health improvement, as evidenced by stress levels and the internal health locus of control [30]. The following sections were evaluated: choosing a healthy diet, avoiding a harmful diet, daily routine, organized physical exercise, and social and mental balance. With this instrument, it was possible to conclude whether the individual was able to maintain adequate life control.

In 1994, Burckhardt et al. [31] developed a specific tool to measure the impact of FM on the functional capacity and quality of life of people who present this pathology: the Fibromyalgia Impact Questionnaire (FIQ). The FIQ assesses the impact of FM on physical

functioning, the ability to perform regular work and, in the case of having a paid job, the degree to which FM has affected this activity, as well as subjective items closely related to the FM clinical profile (pain, fatigue, tiredness, and stiffness) and emotional state (anxiety and depression). Spanish version of the questionnaire was used. To obtain the total score, the different items were normalized, as a result the total score oscillated between 0–80 [32]. A higher score indicates a negative impact of FM on the patient's health.

Lastly, and given the pandemic era we are living in, we decided to include questionnaires that evaluated fear and anxiety towards Coronavirus Disease 2019 (COVID-19): the Coronavirus Anxiety Scale (CAS) and the Fear of COVID-19 Scale (FCV-19S).

The CAS is a brief mental health assessment that can be used to identify cases of dysfunctional anxiety related to COVID-19 [33]. The items measure physiological symptoms that are awakened by information and thoughts related to the coronavirus using a 5-point time-anchored scale (0 = not at all, to 4 = almost every day during the last 2 weeks). The higher the score, the greater the perceived anxiety related to COVID-19. The Spanish version of Caycho-Rodríguez et al. [34] was used.

The FCV-19S is used to identify people with high levels of fear of COVID-19, and perform early psychological interventions [35]. It is a 7-item scale that is scored using a 5-point Likert scale, ranging from 1 (totally disagree) to 5 (totally agree). Total scores can range from 7 to 35, with higher scores indicating greater fear related to COVID-19. The Spanish version of Sánchez-Teruel and Robles Bello [36] was used.

2.3. Procedure

Quality of life and psychological status of patients with FM, with or without codiagnosis of CFS, and with self-reported HPE, were evaluated comparatively, all of this in relation to the RG of the same age range. All participants filled a questionnaire answering about performance of physical activity in the last 3 months. This questionnaire asked participants about intensity, frequency, and type of exercise, as well as their adherence to the program. All types of supervised programs of regular physical exercise for FM patients performed in the 3 months prior to completing the questionnaires, for a minimum of two hours a week on alternate days, were considered as HPE (Table 2). Daily activities could not be evaluated, but participants did not refer to take a walk or bike ride to the job. No differences were found in the professional work activities between the experimental groups (Table 1). All questionnaires were filled by all participants under supervision in December 2020.

Table 2. Percentage of subjects reporting Habitual Physical Exercise.

	RG	FM-Only	FM + CFS Chi-Square (X^2)
Reporting HPE (%)	54.29	28.95 *	53.13 $p < 0.05$ ($X^2 = 0.03$)
***Type of reported HPE*:**			$p > 0.05$ ($X^2 = 0.32$)
- **Walking (%)**	42.1	63.6	58.8
- **Pilates (%)**	15.8	18.2	23.5
- **Yoga (%)**	10.5	9.1	11.8
- **Aquagym (%)**	5.3	9.1	5.9
- **Others (%)**	26.3	___	___

Data are expressed as percentage (%). RG: Reference Group, FM: Fibromyalgia, CFS: Chronic Fatigue Syndrome, HPE: Habitual Physical Exercise, * $p < 0.01$ with respect to both reference and FM + CFS groups (post hoc Z-test).

2.4. Analysis of Data

Values are expressed as mean ± standard error of the mean (SEM). The variables were normally distributed tested by the Kolmogorov–Smirnov normality test. Student's *t*-test was used for comparisons between groups (paired samples). Chi-square independence test and z-test for independent proportions with Bonferoni corrections was used for comparisons between percentages in Tables 1 and 2. Minimum significance level was set at $p < 0.05$. Statistical analysis was performed with the SPSS® Statistics v.27.0 package.

3. Results

3.1. Psychological State and Quality of Life

Figure 2 shows the psychological state and quality of life of our group of women diagnosed with FM. FM patients showed worse values ($p < 0.001$) of depression (Figure 2a), stress (Figure 2b), anxiety (Figure 2c), pain (Figure 2d), fatigue (Figure 2e), impact of fibromyalgia (Figure 2f) and greater fear (Figure 2g) and anxiety towards COVID-19 (Figure 2h) compared to RG.

Once corroborated, as expected, the deterioration in psychological health and quality of life of our population, we wanted to know how the diagnosis of FM, with and without codiagnosed CFS, affects the performance of regular physical activity reported by the study volunteers (Table 2). Paradoxically, the group of patients with FM + CFS presented a higher percentage of women who reported performing HPE, even in the same order of magnitude as the group of healthy women. No differences were found between the experimental groups in the type of HPE reported by the participants. In addition, any correlation between participants reporting HPE and their daily professional work or common comorbidities could be determined.

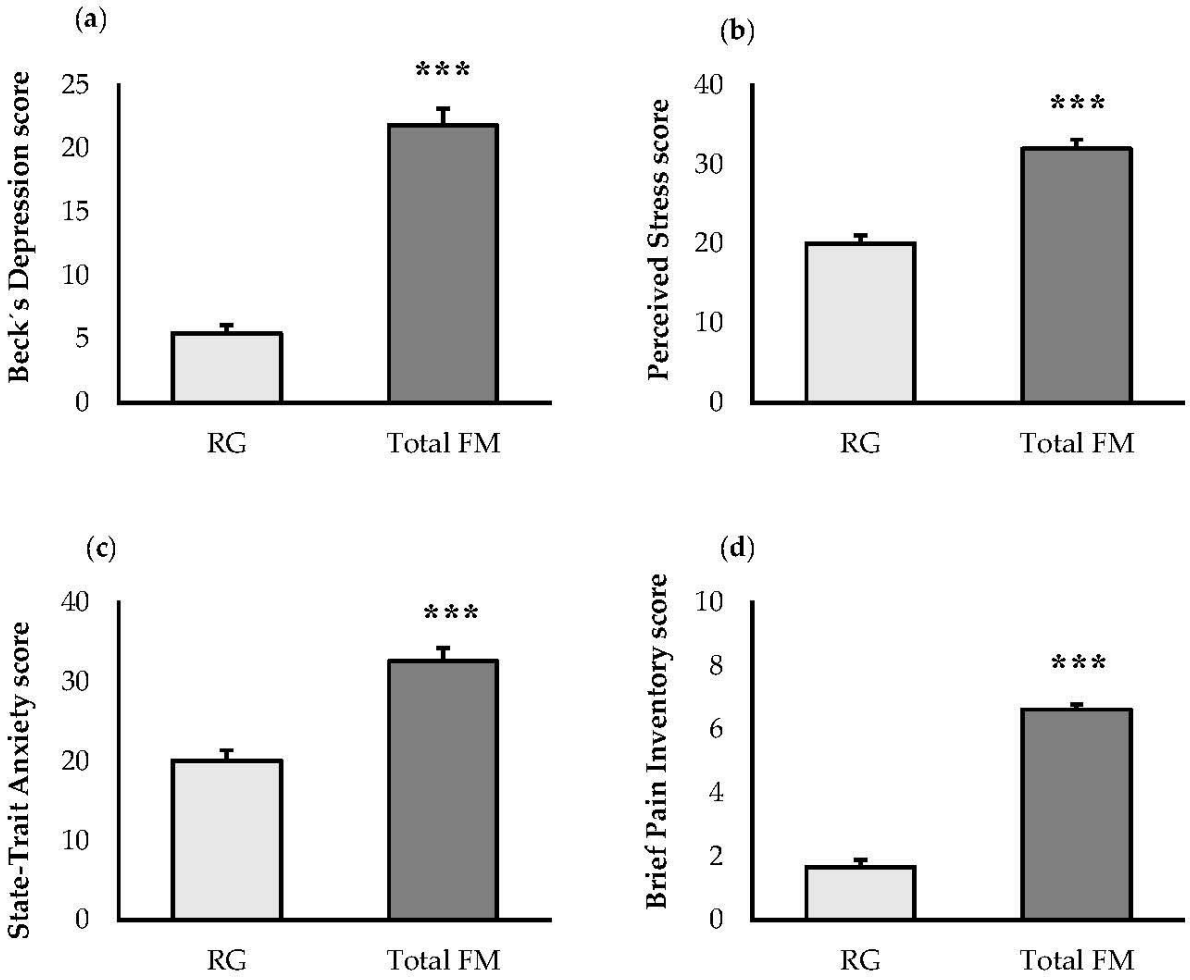

Figure 2. *Cont.*

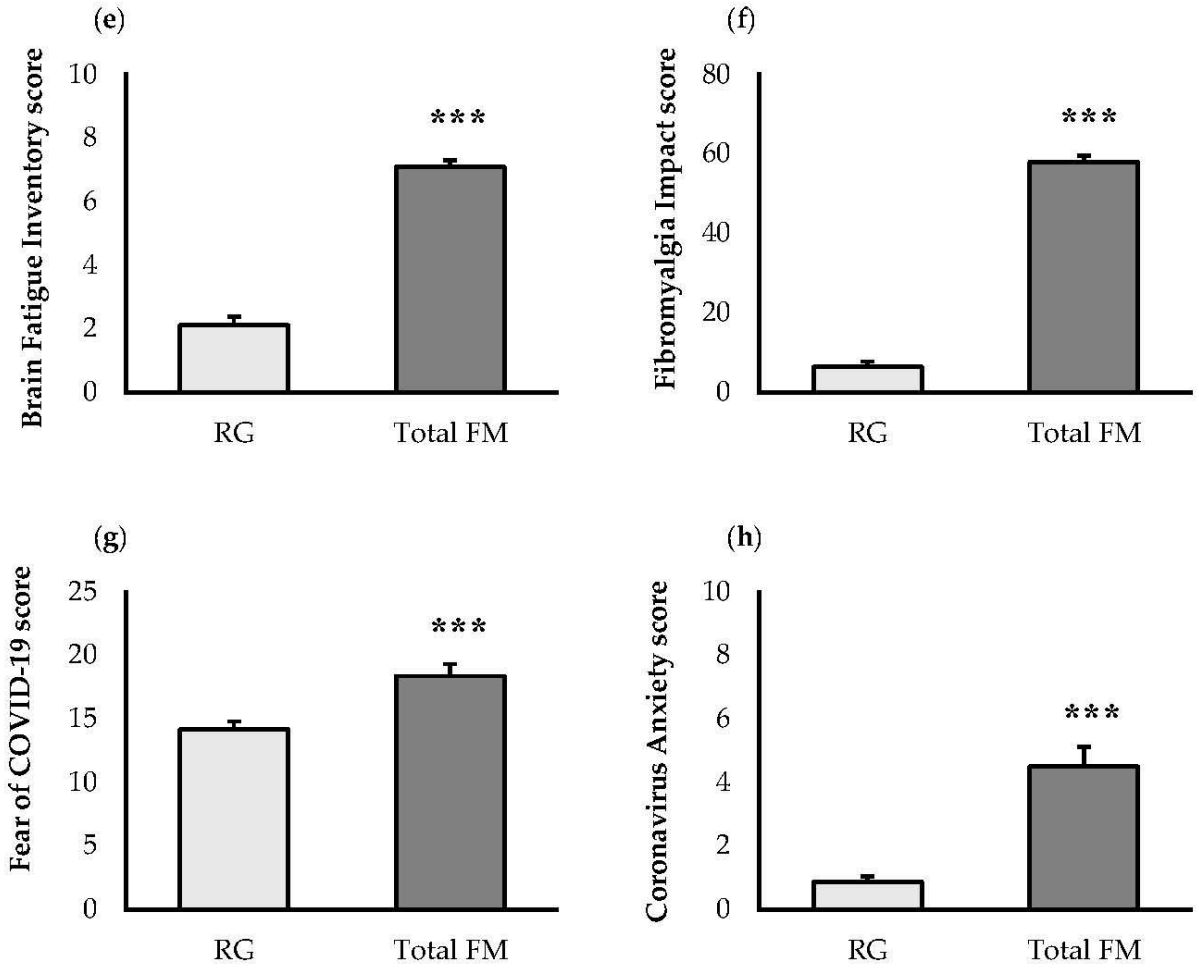

Figure 2. Psychological state and quality of life of FM patients (Total FM, n = 70) compared to an age-matched reference group of "healthy" women (RG, n = 70): perceived levels of (**a**) depression; (**b**) stress; (**c**) anxiety; (**d**) pain; (**e**) fatigue; (**f**) fibromyalgia impact; (**g**) fear of COVID-19; (**h**) coronavirus anxiety. Determinations are expressed by the mean ± SEM of each group. RG: Reference Group, FM: Fibromyalgia. *** $p < 0.001$ with respect to the reference group.

3.2. *Influence of Codiagnosis of CFS and Performance of HPE*

Figure 3 shows the influence of the codiagnosis of CFS and the performance of HPE on the psychological state and mental health of women with FM. The results clearly show that codiagnosis of CFS does not affect ($p > 0.05$) levels of depression (Figure 3a), stress (Figure 3b), state anxiety (Figure 3c) or trait anxiety (Figure 3d). In both groups separately, worse values were manifested again in the evaluated parameters ($p < 0.001$) with respect to the RG. However, patients with FM (both in the presence and in the absence of codiagnosis of CFS) who report performing HPE present significantly ($p < 0.05$) better levels of depression, stress, and anxiety than sedentary ones, even reaching anxiety values that are very close to the RG.

When COVID-19-related anxiety was studied in a specific way (Figure 3e), the behavior followed the same pattern, but it was substantially exacerbated. The intensity of this particular anxiety might be the reason why physical activity did not improve it significantly, although a strong tendency to improvement is observed. When fear was assessed (Figure 3f), the pattern was similar, although in this case, both groups of women (FM-only and FM + CFS groups) who reported performing HPE had lower levels of fear ($p < 0.05$), even almost reaching the level of our RG.

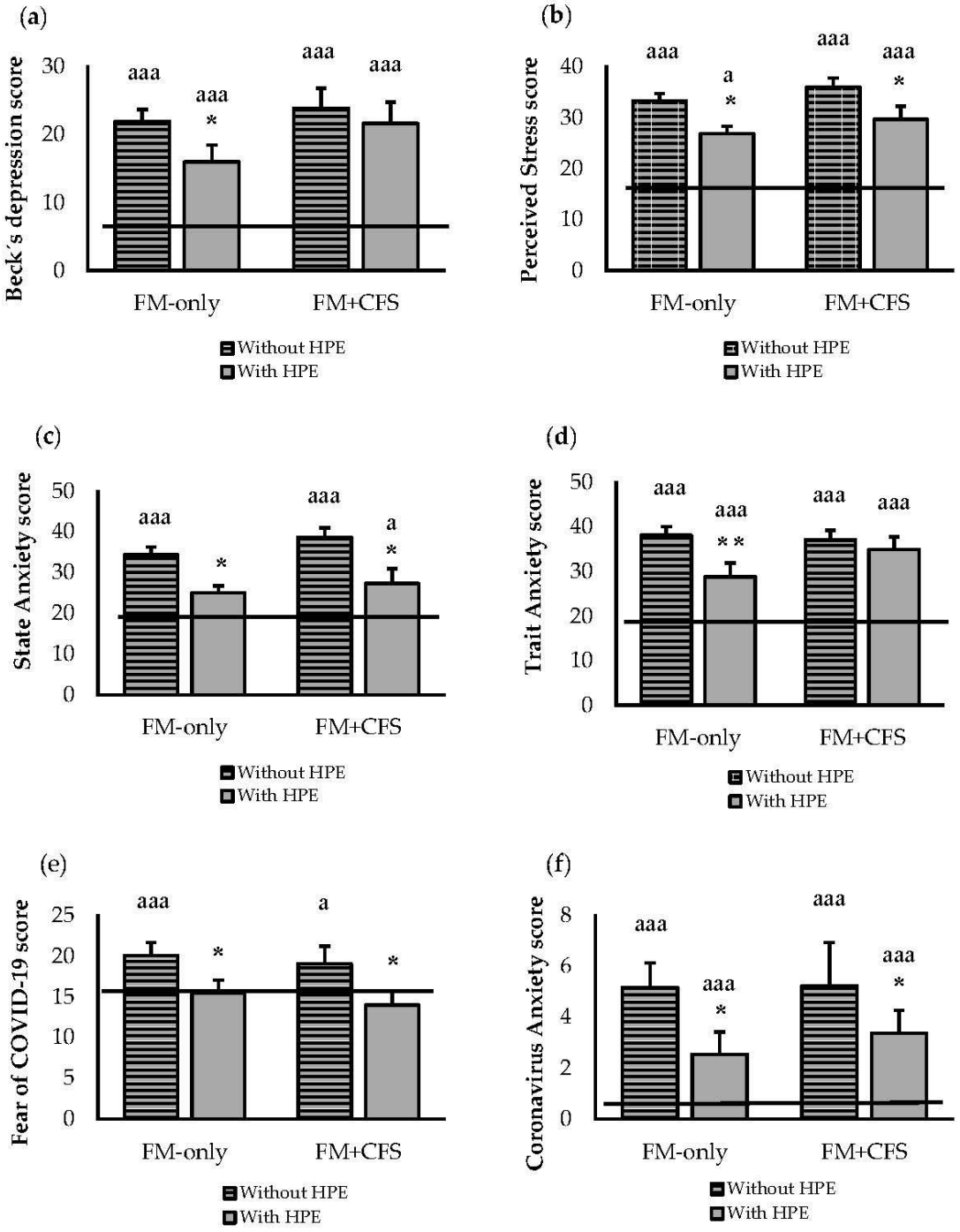

Figure 3. Effect of Habitual Physical Exercise (HPE) in patients diagnosed only with FM (FM-only, n = 38: 27 without HPE and 11 with HPE) and codiagnosed with CFS (FM + CFS, n = 32: 15 without HPE and 17 with HPE), on psychological status and mental health: perceived levels of (**a**) depression; (**b**) stress; (**c**) state anxiety; (**d**) trait anxiety; (**e**) fear of COVID-19 and (**f**) coronavirus anxiety. Horizontal line represents values obtained in the age-matched reference group of "healthy" women without FM. Columns represent the mean ± SEM of each experimental group with or without reported HPE. FM: Fibromyalgia, CFS: Chronic Fatigue Syndrome. * $p < 0.05$, ** $p < 0.01$ with respect to the corresponding group without HPE. ^{a} $p < 0.05$, ^{aaa} $p < 0.001$ with respect to reference group. "Without HPE": participants non reporting HPE; "With HPE": participants reporting HPE.

Figure 4 shows the influence of the codiagnosis of CFS and the performance of HPE on quality of life of our group of patients with FM. The codiagnosis with CFS did not affect quality of life and personal control (Figure 4a), nor pain (Figure 4b) of the patients with FM, although a worsening of all the parameters was observed compared to our RG. Only when comparing the results obtained in the complete FM + CFS group, both sedentary and performing HPE, a statistically significant ($p < 0.01$) greater fatigue was observed compared to the complete group of patients diagnosed with only FM (statistical significance that does not appear in Figure 4c. Patients with FM (both in the presence and in the absence of codiagnosis of CFS) who reported performing HPE presented significantly higher levels of quality of life and personal control ($p < 0.001$) in the only FM group and $p < 0.01$ in the FM + CFS group) than sedentary women, reaching the values of our RG. However, no improvement in fatigue and pain levels was observed in women with FM who reported performing HPE neither in the presence nor in the absence of a codiagnosis of CFS. Finally, only in FM-only group, HPE seem to have a positive influence on the impact of fibromyalgia on daily life ($p < 0.05$) (Figure 4d).

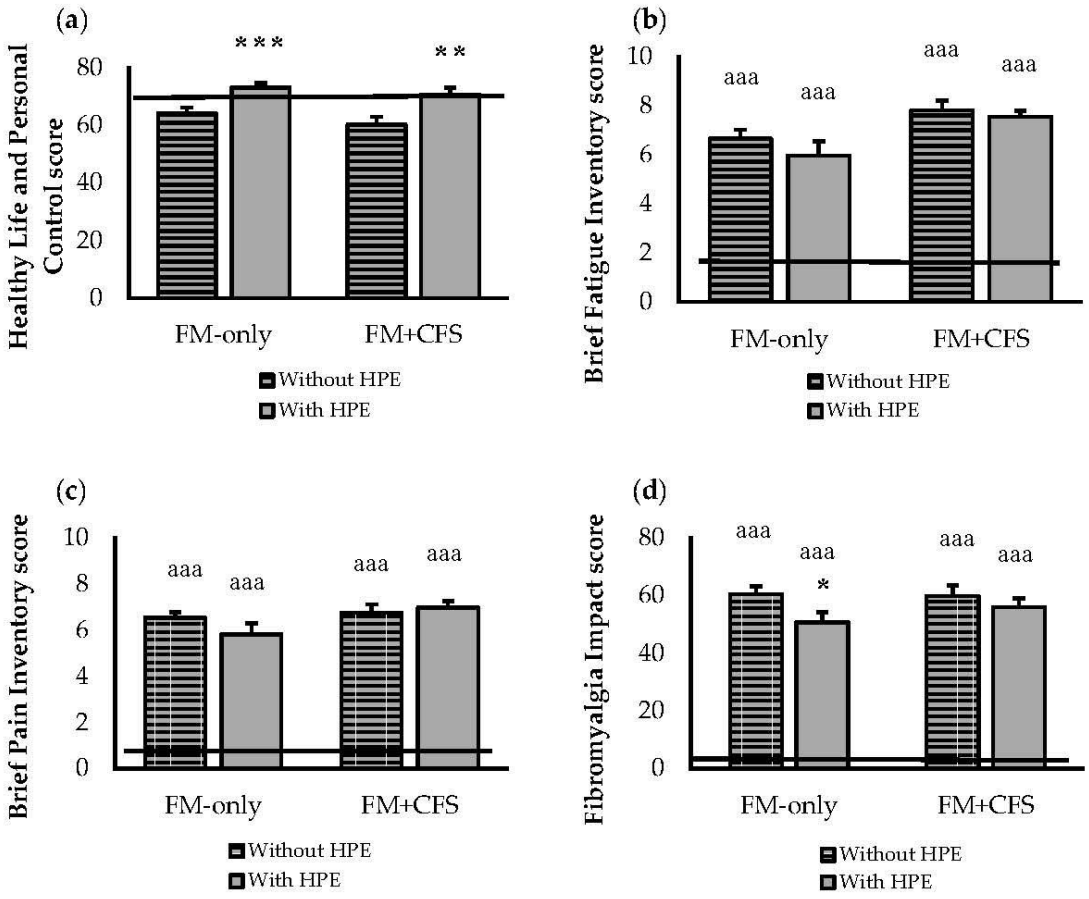

Figure 4. Effect of Habitual Physical Exercise (HPE) in patients diagnosed only FM (FM-only, n = 38: 27 without HPE and 11 with HPE) and codiagnosed with CFS (FM + CFS, n = 32: 15 without HPE and 17 with HPE), on quality of life: perceived levels of (**a**) healthy life and personal control (**b**) fatigue; (**c**) pain and (**d**) fibromyalgia impact. Horizontal line represents values obtained in the age-matched reference group of "healthy" women without FM. Columns represent the mean ± SEM of each experimental group with or without reported HPE. FM: Fibromyalgia, CFS: Chronic Fatigue Syndrome. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ with respect to the corresponding group without HPE. ^{aaa} $p < 0.001$ with respect to reference group. "Without HPE": participants non reporting HPE; "With HPE": participants reporting HPE.

Finally, Table 3 shows the items evaluated in the HLPCQ questionnaire, with their corresponding statistics.

Table 3. Influence of habitual physical exercise (HPE) on the values of the HLPCQ items questionnaire in patients with FM, with and without associated CFS.

	RG		FM-Only		FM + CFS	
Items HLPCQ	**Without HPE**	**With HPE**	**Without HPE**	**With HPE**	**Without HPE**	**With HPE**
Dietary Healthy Choices score	14.89 ± 0.52	16.66 ± 0.61 **	16.48 ± 0.70	19.00 ± 0.90 *	15.94 ± 0.94	18.00 ± 0.79 *
Dietary Harm Avoidance score	9.43 ± 0.89	9.05 ± 0.35	10.56 ± 0.43	11.31 ± 0.49	9.75 ± 0.57	11.94 ± 0.58 **
Daily Routine score	24.26 ± 0.25	24.49 ± 0.66	22.89 ± 1.08	24.92 ± 1.11	21.06 ± 1.71	22.88 ± 1.18
Organized Physical Exercise score	3.37 ± 0.46	6.22 ± 0.25 ***	3.56 ± 0.23	5.62 ± 0.58 **	3.75 ± 0.48	5.24 ± 0.49 **
Social and Mental Balance score	12.91 ± 1.61	13.54 ± 0.47	11.44 ± 0.49	11.69 ± 0.68	10.81 ± 0.56	12.24 ± 0.58 *
Total score	65.53 ± 1.61	70.23 ± 1.42 **	64.89 ± 1.74	72.54 ± 1.74 ***	61.31 ± 2.85	70.29 ± 2.41 **

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ with respect to the corresponding group without HPE. Data are expressed as mean ± SEM. RG: Reference Group, FM: Fibromyalgia, CFS: Chronic Fatigue Syndrome, HLPCQ: Healthy Life and Personal Control Questionnaire. "Without HPE": participants non reporting HPE; "With HPE": participants reporting HPE.

No significant differences in any score corresponding to diet, organized exercise, and social and mental balance were found among participants from the three groups. However, participants reporting HPE improved dietary healthy choices and organized physical exercise scores in the three groups, and social and mental balance only in the FM + CFS group. Total score of HLPCQ improved in participants reporting HPE from the three groups.

4. Discussion

In recent years, our research group has evaluated different immunoneuroendocrine mechanisms underlying the pathophysiology of FM syndrome, clearly observing a neuroimmunoendocrine dysregulation between immune and stress responses that can be improved by physical exercise [5,7,8,37]. These studies have been carried out in patients belonging to different associations from the autonomous community of Extremadura (Spain). The population, due to its socio-sanitary characteristics, lifestyle, and follow-up by the public health system, has already been referred to in many studies as suitable for scientific studies on health and well-being [15,16]. Additionally, in terms of FM, this region is a reference throughout Europe due to the abundance of research, from different approaches, by different groups. However, a practical problem that arises is the need for diagnosis and therapeutic management in primary healthcare and psychological consultations in a simple manner, that is, without expensive clinical analyses and tests evaluating biomarkers of inflammation/stress and biomarkers associated with fatigue and pain mediators. In this context, it is also essential that professionals can address the differential (or similar) management of patients with FM and/or CFS as diagnosed by rheumatologists or internists either jointly or in separate diagnoses.

Although FM and CFS are two different diseases with different diagnostic criteria, there is a considerable overlap between the two as they share many clinical features. From a pathophysiological point of view, it is thought that both conditions are caused by the same alterations involving inflammatory, infectious and/or autoimmune components [38]. Contrary to that described in the work by Faro et al. [39], where the codiagnosis of FM in patients with CFS worsens clinical parameters, fatigue, and quality of life perception, in the present investigation we found more similarities than differences in these two alterations.

Our results showed that the codiagnosis of CFS did not significantly affect the parameters evaluated in patients with FM, and that physical exercise, in these patients as reported in previous investigations from our group in FM patients, is an effective non-pharmacological therapy for the alleviation of some of the dysregulations found in these patients. In the present work, we had a homogeneous group of volunteers in which approximately half of the patients with FM had also been diagnosed with CFS. All of them, as expected, showed worse values in all the measured parameters regarding mental health and impact of FM on quality of life than the group of "healthy" volunteers of the same age range. Nevertheless, HLPCQ, a general questionnaire for evaluating quality of life but not specific for FM patients, did not showed differences between FM patients and the control group. This could reveal that this questionnaire may not be appropriate for evaluating the impact of FM in the quality of life of these patients, although it does reflect the beneficial effects of participation in regular physical exercise programs. Paradoxically, almost half of the volunteers with FM codiagnosed with CFS reported performing regular physical exercise, which is a similar proportion to that in our reference group of control women; moreover, this was a higher percentage than that of the FM-only group. This might be because, although women with diagnosed CFS subjectively report substantial intolerance to exercise, pharmacological treatment is also much more complicated and less effective than in women with FM alone. Therefore, perhaps the group of women with FM and associated CFS, resort to non-pharmacological therapies as the only alternative, hoping for a considerable improvement in their health and quality of life, despite the difficulty that this entails. In fact, regular physical exercise has been well proven to reduce symptoms in patients with FM, by improving both psychological and physiological aspects such as depression, anxiety, body composition, pain and quality of life [40], and immunophysiological biomarkers that underlie the improvement in quality of life [5,7,8,37]. Similarly, in people with CFS, Dannaway et al. [41] reported positive effects of physical exercise through pathways that reverse physical deconditioning or central sensitization to exertion or activity. However, very little has been described about how exercise influences when both syndromes are concomitant. In the present investigation, we observed that the patients who reported performing HPE also presented improvements in almost all of the mental health and quality of life parameters that were evaluated, without a positive or negative influence of the CFS codiagnosis. Therefore, the HPE reported by the patients, which consisted mostly of physical activities with an important social component, had a positive impact on stress, anxiety, and quality of life and personal control in both experimental groups. Personal control values even became closer to the reference levels of control women, but without differences between the experimental groups. That is, codiagnosis with CFS does not, in general, have an effect on the responses to exercise. A differential effect in FM + CFS and FM-only patients was only observed in depression levels in response to exercise, given that only those without CFS improved. These results are in line with those recently reported by Larun et al. [13] since they found that physical exercise did not improve depression in patients with CFS.

We also decided to evaluate anxiety and fear of COVID-19, since one of the main characteristics of women with FM is the lower capacity to handle stressful situations, and it is important to take into account that altered emotional states caused by additional dysregulation of the limbic system in centrally sensitized patients can intensify symptoms of depression and anxiety [42]. Some studies suggest that symptoms from FM patients who have had COVID-19 got worse, as it can be expected due to their hypersensitivity state [43]. Furthermore, a pilot study by Cankurtaran et al. [44] showed that anxiety and fear of COVID-19 worsened the symptoms and mood of FM patients. Therefore, in our study we had to consider whether women who reported performing HPE during the pandemic might experience less anxiety and fear of COVID-19 than more sedentary patients, and whether the codiagnosis with CFS influenced this matter. Results clearly showed that women who reported performing HPE coped better with the pandemic situation, including fear of contracting the disease, all of this at similar levels to those of healthy women of

the same age range. HPE practice encouraged them to go outdoors and continue leading a normal life as far as possible. This finding is supported by Martins et al. [45], who also pointed out that a high proportion of FM patients quit exercising during the COVID-19 pandemic, resulting in an aggravation of the impact of FM during that period.

On the other hand, and paradoxically, although pain is the differentiating, hallmark symptom in FM patients, whereas in CFS patients fatigue is, both experimental groups reported equal pain, but the FM group codiagnosed with CFS reported significantly greater fatigue. However, no differences were observed between the women of the two experimental groups who reported performing physical activity and the sedentary women in any of the indicated parameters including pain. Other studies, using the transcutaneous electrical nerve stimulation (TENS) (FAST) technique in patients with FM, have not observed an improvement in pain either, finding an insignificant relationship between pain and physical activity, although FM patients who performed physical activity did find an improvement in fatigue [46].

Since this research is not an interventional study, it presents the limitations corresponding to an observational study in which it was not possible to control, principally, neither the intensity and type of exercise programs nor the prescribed medication, particularly antidepressant drugs and other possible self-medications. That is why future research focusing whether the health perceived by FM patients corresponds to objective immunoneuroendocrine biomarkers and objective levels of physical activity evaluated through accelerometry will be essential to propose more objective and personalized therapeutic strategies.

5. Conclusions

Therefore, we can conclude that codiagnosis with CFS does not negatively affect the already-impaired psychological state and quality of life of FM patients. In addition, HPE has a positive effect on the psychological state and quality of life of patients with FM, without a great influence of the codiagnosis of CFS.

Finally, FM and CFS are still diseases that represent a great challenge to modern medicine, since the etiopathogenesis, diagnosis, and possible therapies are not yet fully clarified. However, we propose that an integrated approach with a realistic individualized therapeutic plan is important in these two conditions and even more so when they occur together, which is in most cases. Further research is crucial, focusing not only on the underlying mechanisms, but also on improvements in exercise-based therapeutic aids that mitigate possible neuroimmunoendocrine dysregulations and also mood disorders; all of this in order to achieve an easier handling of these conditions in the context of psychological care and primary healthcare.

Author Contributions: Conceptualization, E.O. (Eduardo Ortega) and M.D.H.; methodology, E.O. (Eduardo Otero), M.D.H., I.G. and L.M.-C.; formal analysis, E.O. (Eduardo Otero) and M.d.C.N.; investigation, M.D.H., E.O. (Eduardo Ortega), I.G., L.M.-C., M.d.C.N. and E.O. (Eduardo Otero); writing—original draft preparation, M.D.H. and E.O. (Eduardo Otero); writing—review and editing, M.D.H., I.G. and E.O. (Eduardo Ortega); supervision, E.O. (Eduardo Ortega); project administration, E.O. (Eduardo Ortega); funding acquisition, E.O. (Eduardo Ortega). All authors have read and agreed to the published version of the manuscript.

Funding: This research was partially funded by the Gobierno de Extremadura- Fondo Europeo de Desarrollo Regional, Spain (GR21079) and by aid to promote the hiring of research support staff in the Autonomous Community of Extremadura (TE-0027-19).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Bioethics Committee of University of Extremadura, Spain (N° Reg. 73/2021, 09/06/2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The raw data supporting the conclusions of the manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

Acknowledgments: We are grateful to the associations of Fibromyalgia and Chronic Fatigue Syndrome belonging to the community of Extremadura: EXISTIMOS (Badajoz, Spain), AFIBRODON (Don Benito, Badajoz, Spain) and AFIBROEX (Cáceres, Spain).

Conflicts of Interest: The authors declare no conflict of interest.

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Publicación 2.

Otero, E., Gálvez, I., Ortega, E.*, e Hinchado, M. D. (2023). Influence of Chronic Fatigue Syndrome Codiagnosis on the Relationship between Perceived and Objective Psychoneuro-Immunoendocrine Disorders in Women with Fibromyalgia. *Biomedicines*, 11(5), 1488. https://doi.org/10.3390/biomedicines11051488

Título: "*Influencia Del Codiagnóstico del Síndrome de Fatiga Crónica en la Relación Entre los Trastornos Psiconeuro-inmunoendocrinos Percibidos y Objetivos en Mujeres con Fibromialgia"*

Resumen

Aunque el síntoma predominante en la fibromialgia (FM) es el dolor muscular, y la fatiga en el síndrome de fatiga crónica (SFC), el diagnóstico diferencial es muy difícil. Esta investigación estudia los trastornos psiconeuroinmunoendocrinos de las pacientes con FM y determina si un diagnóstico previo de SFC les afecta. Mediante parámetros objetivos de acelerometría, se estudian los niveles de actividad física/sedentarismo en relación con la fatiga, así como si los niveles percibidos de estrés, ansiedad y dolor se corresponden con biomarcadores objetivos, todo ello respecto a un grupo de referencia (GR) de mujeres sin FM. Las pacientes con FM presentan un peor estado psicológico y calidad de vida percibida que las del GR. Estos resultados percibidos son coherentes con el deterioro de los niveles objetivos de un estilo de vida sedentario, niveles sistémicos más altos de cortisol y noradrenalina, y niveles más bajos de serotonina. Sin embargo, las pacientes de FM con un diagnóstico previo de SFC presentaron niveles sistémicos más bajos de IL-8, cortisol, oxitocina, y niveles más altos de adrenalina y serotonina que las pacientes con FM sin diagnóstico de SFC. En conclusión, mientras que en los parámetros de salud percibida no se detectan diferencias, cuando se utilizan parámetros neuroinmunoendocrinos objetivos relacionados con el estrés, la inflamación, el dolor y la fatiga, las personas con SFC podrían estar sobrediagnosticadas de FM. Esto refuerza la necesidad de una evaluación objetiva de biomarcadores en estos pacientes para una mejor discriminación diagnóstica entre ambos síndromes.

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Influence of Chronic Fatigue Syndrome Codiagnosis on the Relationship between Perceived and Objective Psychoneuro-Immunoendocrine Disorders in Women with Fibromyalgia

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Abstract: Although the predominant symptom in fibromyalgia (FM) is muscle pain, and fatigue in chronic fatigue syndrome (CFS), differential diagnosis is very difficult. This research investigates the psychoneuroimmunoendocrine disorders of FM patients and ascertains whether a previous CFS diagnosis affected them. Through accelerometry objective parameters, physical activity/sedentarism levels in relation to fatigue are studied, as well as whether perceived levels of stress, anxiety, and pain correspond to objective biomarkers, all of these with respect to a reference group (RG) of women without FM. FM patients have a worse psychological state and perceived quality of life than those with RG. These perceived outcomes are consistent with impaired objective levels of a sedentary lifestyle, higher systemic levels of cortisol and noradrenaline, and lower levels of serotonin. However, FM patients with a previous CFS diagnosis had lower systemic levels of IL-8, cortisol, oxytocin, and higher levels of adrenaline and serotonin than FM patients without diagnosed CFS. In conclusion, while perceived health parameters do not detect differences, when objective neuroimmunoendocrine parameters related to stress, inflammation, pain, and fatigue are used, people with CFS could be overdiagnosed with FM. This reinforces the need for objective biomarker assessment of these patients for better diagnostic discrimination between both syndromes.

Keywords: fibromyalgia; chronic fatigue syndrome; accelerometry; inflammation; stress; cortisol; IL-8; catecholamines; serotonin; oxytocin

Citation: Otero, E.; Gálvez, I.; Ortega, E.; Hinchado, M.D. Influence of Chronic Fatigue Syndrome Codiagnosis on the Relationship between Perceived and Objective Psychoneuro-Immunoendocrine Disorders in Women with Fibromyalgia. *Biomedicines* **2023**, *11*, 1488. https://doi.org/10.3390/biomedicines11051488

Academic Editor: Ryan S. D'Souza

Received: 17 April 2023
Revised: 12 May 2023
Accepted: 18 May 2023
Published: 20 May 2023

1. Introduction

Fibromyalgia (FM) is defined as a syndrome characterized by chronic widespread pain associated with other physical disorders such as hyperalgesia, allodynia, and fatigue [1]. Prevalence of FM is approximately 6.3%, and it is considerably more frequent in women (90% of cases), according to the World Fibromyalgia Association. Differential diagnosis of FM is a serious problem, because it is often associated with different pathologies that present similar symptoms, both perceived symptoms and those assessed through objective biomarkers. One of the most frequently associated syndromes is chronic fatigue syndrome (CFS), defined as disabling fatigue of 6 months or more duration, which is also related to other disorders such as psychological disturbances and unrefreshing sleep [2].

Although the aetiology of FM is not completely established, both abnormalities in the function of the autonomic nervous system and neuroimmunoendocrine alterations have been implicated in its pathogenesis. There are many studies through which our

research group has established a clear relationship between FM and dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis [3–5]. This alteration strongly contributes to persistent pain and affective distress; it may be mediated by pro-inflammatory cytokines, chemokines, and stress mediators, such as systemic IL-8 and cortisol [3,6–9]. Additionally, in CFS, neuroimmunoendocrine disruption has been reported [10] and potential biomarkers for CFS diagnosis have been reported [11]; however, their validation has been difficult [12,13]. There are also controversies in the possible imbalance of inflammatory cytokines in the case of CFS, where distinctly different cytokine association networks have been reported in healthy individuals [11].

In this context, although both syndromes have a high percentage of comorbidity, limited studies have considered CFS codiagnosis and how it influences quality of life, objective physical activity and fatigue, and the neuroimmunoendocrine status of patients with FM. Therefore, based on the need to find objective biomarkers reflecting the symptoms and perceived health of patients with FM that could help to make a differential diagnosis between the two syndromes, we hypothesized that CFS codiagnosis would negatively influence physical activity and the already dysregulated neuroimmunoendocrine status of patients with FM. The overall aim was to delve into the psychoneuroimmunoendocrine disorders of patients with FM and to test whether a previous CFS diagnosis could affect them, assessing both patients' perception through scientifically validated questionnaires, and objectively through accelerometry and systemic immunophysiological biomarkers of inflammation, stress, and anxiety.

This differential study is justified in the context of the decreased capacity for daily activity that these patients present due to pain and other nervous disorders. We believe that this research could contribute to validating and objectifying the more subjective aspects of patients' quality of life, through objective biomarkers, and thus improve the differential diagnosis of these syndromes.

2. Materials and Methods

2.1. Participants and Experimental Design

This study was carried out with 34 patients (total group of FM patients), all aged between 40 and 65 years. They belonged to the FM associations of Extremadura, an autonomous community whose population is very homogeneous in terms of lifestyle. The majority of the population are covered by the Spanish National Health System. Moreover, this region is a reference region in health research in Spain [14,15], particularly for the study of fibromyalgia and of the effects of exercise internationally [16]. A total of 17 of these FM patients had a previous CFS diagnosis (FM + CFS group) and the remaining 17 were FM patients without a CFS codiagnosis (FM group). A group of 11 women of the same age range constituted the reference group of "healthy" women not diagnosed with FM, CFS or any other inflammatory or rheumatic pathology, or any condition involving depression, anxiety and/or pain (RG group).

The selected patients met the following inclusion criteria: (a) FM diagnosis with or without a previous CFS codiagnosis by rheumatologists or internal medicine professionals according to the American College of Rheumatology (ACR) diagnostic criteria for FM patients [1], and the Fukuda and co-workers criteria for CFS patients [2]; (b) age 40 to 65 years; (c) not having a diagnosis of depression; (d) not having multiple chemical sensitivity; (e) not having performed scheduled physical activity in the previous two months or during the accelerometry tests; (f) not taking corticosteroids or anti-cytokine therapy.

At the first phase of the study, anthropometric characteristics, employment status and body composition were assessed (Table 1). Medication history was very diverse in each patient; however, the vast majority of women had a prescription for different types of anti-inflammatory and analgesic drugs (e.g., ibuprofen, dexketoprofen, paracetamol, tramadol), excluding patients with prescribed corticosteroid treatments or anti-cytokine therapies.

Table 1. Anthropometric characteristics, employment status, and body composition of the participants.

	Reference Group (n = 11)	Total FM Patients (n = 34)	FM (n = 17)	FM + CFS (n = 17)	Statistical Significance
Gender (%)	Women (100%)	Women (100%)	Women (100%)	Women (100%)	
Ethnic group (%)	White (100%)	White (100%)	White (100%)	White (100%)	
Duration of FM diagnosed (years)		>2	>2	>2	
Age (years)	55.81 ± 2.08	57.84 ± 1.29	57.20 ± 1.84	58.41 ± 1.85	$p > 0.05$
BMI (kg/m^2)	24.62 ± 0.83	27.30 ± 0.91	27.42 ± 1.37	27.19 ± 1.25	$p > 0.05$
Employment status					Chi-Square (X^2) $p > 0.05$ ($X^2 > 0.05$)
Blue collar workers (%)	18.2	20.6	17.6	23.5	
White collar workers (%)	36.4	11.8	11.8	11.8	
Unemployed (%)	36.4	23.5	29.4	17.6	
Medical leave (%)		23.5	23.5	23.5	
Retired (%)	9.1	20.6	17.6	23.5	
Body composition					
Body fat mass (%)	36.70 ± 1.57	39.61 ± 1.50	38.68 ± 1.54	40.43 ± 2.51	$p > 0.05$
Bone mass (kg)	2.1 ± 0.05	2.11 ± 0.04	2.12 ± 0.05	2.11 ± 0.05	$p > 0.05$
Body water (%)	43.67 ± 1.00	42.55 ± 0.63	42.72 ± 0.97	42.40 ± 0.86	$p > 0.05$
Muscle mass (kg)	39.00 ± 0.80	39.48 ± 1.50	39.64 ± 0.86	39.34 ± 1.06	$p > 0.05$
Visceral fat index	7.31 ± 0.71	9.13 ± 0.47 [a]	9.10 ± 0.58 [a]	9.14 ± 0.79 [a]	$p < 0.05$

Data are expressed as mean ± SEM. RG: Reference Group, FM: Fibromyalgia, CFS: Chronic Fatigue Syndrome, [a] $p < 0.05$ with respect to reference group (Student's t-test).

Subsequently, each participant completed the questionnaires given to them individually, in a supervised manner and with the corresponding indications as to how and when to complete them. These questionnaires were finally collected for the quantification of the values and their subsequent analysis. Afterwards, each participant was given an accelerometer, which enables the objective assessment of physical activity and sedentary lifestyle. All volunteers were required to wear it on the wrist of their non-dominant hand. In order to obtain more information and given that the pace of life varies depending on whether it is a weekend or a weekday, the study was conducted over seven days. After the last day, the accelerometers were collected for further processing of the recorded data using the "Actilife v.6" software (ActiGraph, LLC., Pensacola, FL, USA), and blood samples were taken for determination of neuroimmunoendocrine biomarkers by ELISA.

2.2. *Body Composition Measurements; Bioimpedance Analysis*

The BIA TANITA DC-360 digital scale (manufactured by Tanita in Tokyo, Japan) was employed to evaluate body composition. The scale's measurement frequencies ranged from 6.25 kHz to 50 kHz and yielded data on several parameters including body fat mass (%), bone mass (kg), muscle mass (kg), body water (%) and visceral fat index. The BMI was computed using the weight/height formula expressed in kg/m^2. The participants were assessed while fasting, wearing light clothing, and with bare feet.

2.3. *Subjective Quality of Life*

The Spanish version of Beck's Depression Inventory developed by Sanz and coworkers [17] was used to determine the presence of signs of depression. Higher scores are associated with greater signs of depression and according to the final score, perceived depression can be classified as: mild (10–19), moderate (20–30), or severe (>30) [18].

The Spanish version of the Perceived Stress Scale (PSS) developed by Remor was used to determine perceived stress levels. It is composed of 14 items with a Likert scale response format, where a higher total score corresponds to a higher level of perceived stress [19,20].

The State-Trait Anxiety Inventory (STAI) is composed of two subscales: state anxiety (transient emotional condition) and trait anxiety (relatively stable characteristic of anxiety proneness) [21]. Each subscale is composed of a total of 20 items in a Likert-type response system. As in the previous questionnaires, higher scores indicate a higher anxiety state. In the present study, the Spanish version of Buela-Casal and Guillén-Riquelme [22] was used.

The Brief Pain Inventory (BPI) and the Brief Fatigue Inventory (BFI) are two self-administered questionnaires designed to assess perceived pain and fatigue, respectively [23,24]. Both consist of two basic magnitudes: intensity and interference scored on scales from 0 "no pain/fatigue" to 10 "worst pain/fatigue". Higher scores correlate directly with a higher perception of pain and fatigue. The Spanish version of the BPI by Badía and co-workers [25] and the Spanish version of the BFI by Valenzuela and co-workers [26] were used.

In order to detect and quantify lifestyle patterns that reflect health improvement and adequate life control, the Healthy Lifestyle and Personal Control Questionnaire (HLPCQ) was used. This questionnaire is composed of several items: choice of a healthy diet, avoidance of a harmful diet, daily routine, organized physical exercise and social and mental balance [27].

The Spanish version of the Fibromyalgia Impact Questionnaire (FIQ) by Rivera and González [28] was used to assess the impact of FM on physical functioning, the ability to perform usual work and the degree to which FM has affected this activity, as well as subjective items closely related to the clinical profile of FM (pain, fatigue, tiredness and stiffness) and emotional state (anxiety and depression) [29]. To obtain the total score, the different items were normalized; therefore, the total score ranged from 0–80 [28]. A higher score indicates a negative impact of FM on the patient's health.

Finally, questionnaires aimed at assessing fear and anxiety about Coronavirus Disease 2019 (COVID-19): Coronavirus Anxiety Scale (CAS) and COVID-19 Fear Scale-19 (FCV-19S) were included. The CAS is a brief mental health assessment that can identify cases of dysfunctional anxiety related to COVID-19 [30]. The Spanish version of Caycho-Rodriguez and co-workers was used [31]. The FCV-19S identifies individuals with high levels of fear of COVID-19 [32]. In the present study, the Spanish version of Sánchez-Teruel and Robles Bello was used [33]. In both questionnaires, a higher score is interpreted as higher anxiety and fear of COVID-19.

2.4. Determination of Objective Levels of Physical Activity and Sedentary Lifestyle

The Actigraph Wgt3x—BT accelerometer was used for the objective determination of physical activity/sedentary levels. This model records the change in acceleration of the center of mass in three planes of motion (x, y, z) and converts them into a quantifiable digital signal called counts. Therefore, counts are units of motion, and each count record is summed and stored in the accelerometer's memory in a configurable period called epoch [34]. In our study, we used an epoch of one minute.

Subsequently, the following parameters were analyzed: count, maximum and average duration of activity and sedentary bouts, and prediction of the metabolic rate through the METs (Metabolic Equivalent of Task) using the algorithm established by Freedson and co-workers [35] through the "Actilife" software (ActiGraph, LLC, Pensacola, FL, USA).

2.5. Blood Collection and Serum Isolation

On the same day of the actigraphic device collection, blood samples were collected from fasting subjects at 08:00 and placed in collection tubes for serum isolation, where they were kept for 15–20 min at room temperature. The serum was centrifuged at 1800× *g* for 15 min. Serum samples were gradually refrigerated at −20 °C as they were obtained. Finally, the samples were stored at −80 °C until further analysis.

2.6. Determination of Neuroimmunoendocrine Markers

Serum concentrations of cortisol (DetectX®, ArborAssays, Ann Arbor, MI, USA), dehydroepiandrosterone (DHEA) (Demeditec Diagnostic GmbH, Kiel, Germany), serotonin (Reddot Biotech. Inc. Katy, TX, USA), oxytocin (CloudClone Corp. Katy, TX, USA), adrenaline and noradrenaline (Demeditec Diagnostic GmbH, Kiel, Germany), interleukin-8 (IL-8) and interleukin-10 (IL-10) (Diaclone SAS, Biotech. Inv. Group, Besancon Cedex, France) were measured using commercial ELISA kits.

2.7. Statistical Analysis

The values are expressed as mean ± SEM. Normality of the variables was checked via the Shapiro–Wilk test, followed by Student's *t*-test for normally distributed samples or Mann–Whitney test for nonparametric samples. Chi-square independence test was used for comparisons between qualitative variables expressed as a percentage. The minimum level of significance was set at $p < 0.05$. Statistical analysis was performed with the SPSS® Statistics v.27.0 package (IBM Corp., Armonk, NY, USA).

3. Results

Firstly, as stated in Table 1, all participants were white women and had been diagnosed with FM (with or without previous CFS diagnosis) for more than two years. Regarding work status, we can see that there were no significant differences between any of the experimental groups. No significant differences were found in age, BMI, fat mass (%), bone mass (kg), body water (%), muscle mass (kg). However, FM patients both with and without CFS codiagnosis had a significantly higher visceral fat index compared to the reference group ($p < 0.05$).

3.1. Psychological Status and Quality of Life

Table 2 shows the psychological status and quality of life. The total group of FM patients showed worse levels ($p < 0.001$) of depression, stress, anxiety, pain, fatigue, impact of fibromyalgia, as well as worse levels ($p < 0.01$) of anxiety and fear towards COVID-19, with respect to the reference group. We can also observe that CFS codiagnosis does not affect FM patients' already-impaired psychological state and perceived quality of life. Higher values ($p < 0.05$) for perceived fatigue were found in FM patients with a previous CFS diagnosis.

Table 2. Psychological state and quality of life.

	RG	Total FM Patients	FM	FM + CFS
Beck's Depression score	6.00 ± 1.37	20.68 ± 2.21 ^{aaa}	17.15 ± 3.12 ^{aa}	23.99 ± 2.97 ^{aaa}
Perceived Stress score	20.09 ± 2.60	31.03 ± 1.79 ^{aaa}	28.60 ± 2.57 ^{aa}	33.30 ± 2.41 ^{aaa}
State-Trait Anxiety score	15.18 ± 2.29	35.65 ± 2.01 ^{aaa}	34.42 ± 2.99 ^{aaa}	36.80 ± 2.79 ^{aaa}
Healthy Life and Personal Control score	70.72 ± 2.36	66.75 ± 1.90	66.35 ± 1.60	67.13 ± 3.43
Brief Pain Inventory score	1.31 ± 0.48	6.12 ± 0.25 ^{aaa}	5.76 ± 0.38 ^{aaa}	6.46 ± 0.33 ^{aaa}
Brief Fatigue Inventory score	1.46 ± 0.47	6.75 ± 0.33 ^{aaa}	6.13 ± 0.53 ^{aaa}	7.33 ± 0.36 ^{aaa} *
Fibromyalgia Impact score	3.5 ± 1.48	54.25 ± 2.41 ^{aaa}	54.90 ± 3.98 ^{aaa}	53.70 ± 3.04 ^{aaa}
Fear of COVID-19 score	12.54 ± 1.39	17.62 ± 1.35 ^{aa}	17.92 ± 2.18 ^{a}	17.33 ± 1.72 ^{a}
Coronavirus Anxiety score	0.18 ± 0.12	4.41 ± 1.01 ^{aaa}	4.21 ± 1.49 ^{aa}	4.60 ± 1.43 ^{aa}

Data are expressed as mean ± SEM. RG: Reference Group, FM: Fibromyalgia, CFS: Chronic Fatigue Syndrome, ^{a} $p < 0.05$, ^{aa} $p < 0.01$, ^{aaa} $p < 0.001$ with respect to reference group. * $p < 0.05$ with respect to FM group (Student's *t*-test).

3.2. Neuroimmunoendocrine Biomarkers

Table 3 shows the systemic concentrations of neuroimmunoendocrine biomarkers in the total group of FM patients compared to the reference group. No significant differences were found in serum levels of IL-8, IL-10 (represented as the percentage of the values above the Lower Limit of Detection (LLD)), DHEA, adrenaline, and oxytocin in total FM patients with respect to the reference group. Nevertheless, higher levels of cortisol ($p < 0.05$), noradrenaline ($p < 0.05$), and lower levels of serotonin ($p < 0.05$) were found in total FM patients.

Table 3. Serum levels of neuroimmunoendocrine biomarkers.

	RG	Total FM Patients
IL-8 (pg/mL)	23.04 ± 3.35	26.24 ± 1.91
IL-10 (>LLD)	9.09%	15.62%
Cortisol (pg/mL)	121,848.21 ± 15,010.97	158,072.90 ± 1413.34 [a]
DHEA (ng/mL)	5.41 ± 0.88	4.48 ± 0.52
Noradrenaline (pg/mL)	168.48 ± 8.42	188.19 ± 6.33 [a]
Adrenaline (pg/mL)	32.32 ± 4.17	33.31 ± 1.46
Serotonin (ng/mL)	271.07 ± 79.18	156.60 ± 14.29 [aa]
Oxytocin (pg/mL)	1248.81 ± 120.08	1447.08 ± 93.56

Data are expressed as mean ± SEM. RG: Reference Group, FM: Fibromyalgia, IL-8: Interleukine-8, IL-10: Interleukine-10, LLD: Low Limit of Detection, DHEA: Dehydroepiandrosterone [a] $p < 0.05$, [aa] $p < 0.01$ with respect to reference group (Student's *t*-test).

3.2.1. Influence of CFS Codiagnosis in FM Patients: Serum Levels of IL-8 and IL-10

FM patients with a previous CFS diagnosis had a significantly lower concentration of IL-8 than patients without CFS ($p < 0.01$; Figure 1). No significant differences in serum levels of IL-8 were found between FM patients with a previous CFS diagnosis and the reference group. However, FM patients without CFS presented higher serum IL-8 levels compared to the reference group ($p < 0.05$), which were also above the reference value (>29 pg/mL) obtained in numerous studies in FM patients of our research group [3–5].

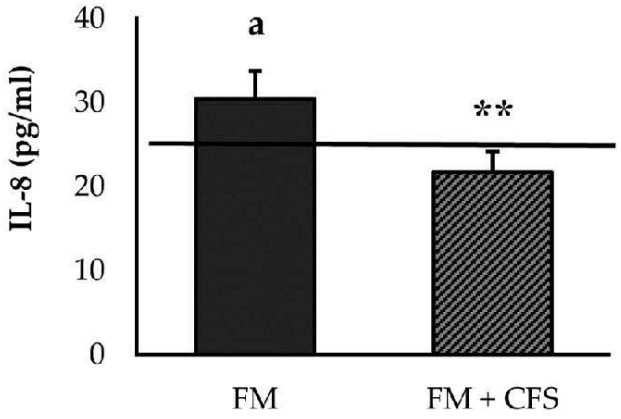

Figure 1. Serum levels of IL-8 in FM patients with (FM + CFS, $n = 17$) or without (FM, $n = 17$) CFS diagnosis. The horizontal line represents values obtained in the age-matched reference group of "healthy" women. Columns represent the mean ± SEM of independent assays performed in duplicate for each participant. IL-8: Interleukine-8, FM: Fibromyalgia, CFS: Chronic Fatigue Syndrome. ** $p < 0.01$ with respect to FM group. [a] $p < 0.05$ with respect to the reference group. (Student's *t*-test).

As explained previously, the results related to IL-10 were determined as the percentage of patients with serum levels of IL-10 above the LLD. No significant differences were found

between both experimental groups (13.3% > LLD in FM patients versus 17.6% > LLD in FM patients with codiagnosis of CFS), and with respect to the reference group.

3.2.2. Influence of CFS Codiagnosis in FM Patients: Serum Levels of Cortisol and DHEA

Figure 2 represents the systemic concentrations of stress-related hormones cortisol (Figure 2a) and DHEA (Figure 2b). Significantly lower serum levels of cortisol were found in FM patients with previous CFS diagnosis compared to FM patients without CFS ($p < 0.05$). Compared to the reference group, no significant differences in serum levels of cortisol were found in FM patients with a previous CFS diagnosis; however, significantly higher serum levels of cortisol were found in FM patients without a CFS diagnosis ($p < 0.01$). Regarding serum levels of DHEA, no significant differences were found in FM patients with or without CFS codiagnosis. No significant differences were found in either experimental group with respect to the reference group.

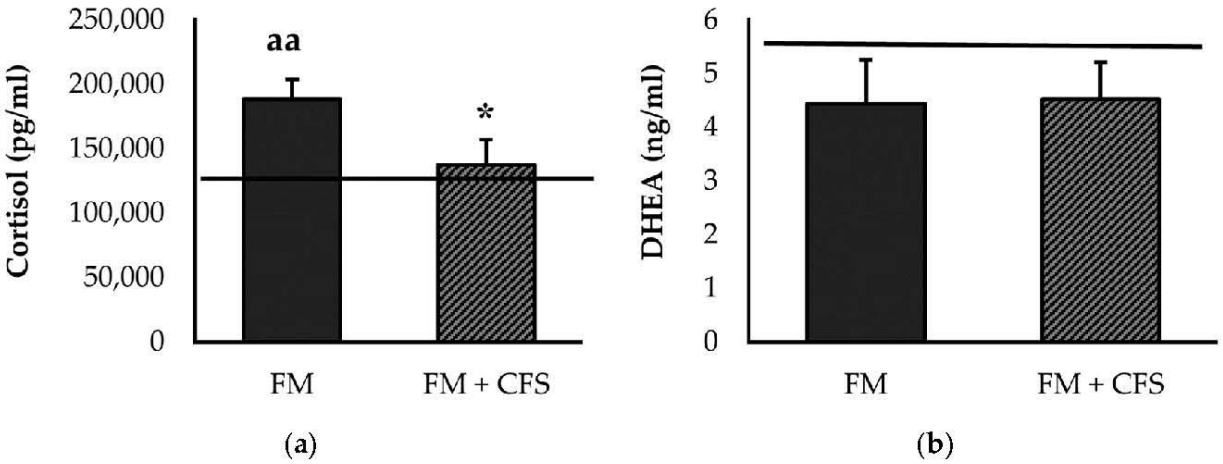

Figure 2. Serum levels of cortisol (**a**) and DHEA (**b**) in FM patients with (FM + CFS, $n = 17$) or without (FM, $n = 17$) CFS diagnosis. The horizontal line represents values obtained in the age-matched reference group of "healthy" women. Columns represent the mean ± SEM of independent assays performed in duplicate for each participant. DHEA: Dehydroepiandrosterone, FM: Fibromyalgia, CFS: Chronic Fatigue Syndrome. * $p < 0.05$ with respect to FM group. ^{aa} $p < 0.01$ with respect to the reference group. (Mann–Whitney *u*-test).

3.2.3. Influence of CFS Codiagnosis in FM Patients: Serum Levels of Noradrenaline and Adrenaline

Figure 3 shows serum concentrations of noradrenaline (Figure 3a) and adrenaline (Figure 3b). No significant differences were found in serum levels of noradrenaline between FM patients with or without CFS codiagnosis. However, significantly higher serum concentrations of noradrenaline were found only in FM patients without a CFS codiagnosis with respect to the reference group ($p < 0.05$).

On the other hand, FM patients with CFS codiagnosis had significantly higher serum adrenaline values compared to FM patients without CFS (v < 0.05). No significant differences were found in the serum levels of adrenaline in FM patients with or without CFS with respect to the reference group.

3.2.4. Influence of CFS Codiagnosis in FM Patients: Serum Levels of Serotonin and Oxytocin

Serum levels of serotonin (Figure 4a) and oxytocin (Figure 4b) are depicted in Figure 4. FM patients with CFS codiagnosis presented significantly higher serum levels of serotonin than FM patients without CFS ($p < 0.01$). In contrast, patients with CFS codiagnosis showed serum serotonin levels that were very close to those of our reference group, without significant differences. However, FM patients without CFS codiagnosis showed significantly lower serum levels of serotonin with respect to the reference group ($p < 0.01$).

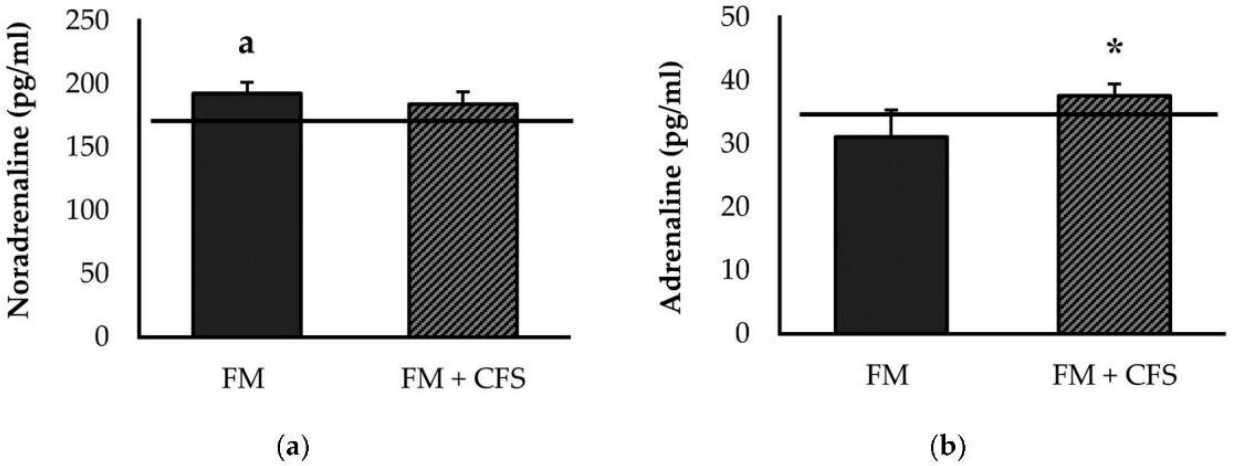

Figure 3. Serum levels of noradrenaline (**a**) and adrenaline (**b**) in FM patients with (FM + CFS, $n = 17$) or without (FM, $n = 17$) CFS diagnosis. The horizontal line represents values obtained in the age-matched reference group of "healthy" women. Columns represent the mean ± SEM of independent assays performed in duplicate for each participant. FM: Fibromyalgia, CFS: Chronic Fatigue Syndrome. * $p < 0.05$ with respect to FM group. [a] $p < 0.05$ with respect to the reference group. (Student's *t*-test).

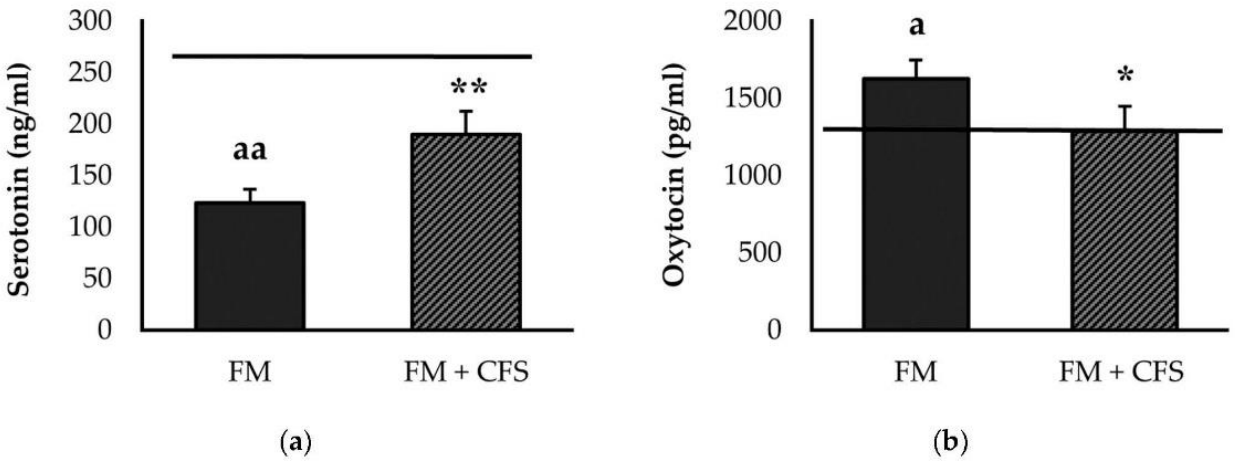

Figure 4. Serum levels of serotonin (**a**) and oxytocin (**b**) in FM patients with (FM + CFS, $n = 17$) or without (FM, $n = 17$) CFS diagnosis. The horizontal line represents values obtained in the age-matched reference group of "healthy" women. Columns represent the mean ± SEM of independent assays performed in duplicate for each participant. FM: Fibromyalgia, CFS: Chronic Fatigue Syndrome. * $p < 0.05$, ** $p < 0.01$, with respect to FM group. [a] $p < 0.05$, [aa] $p < 0.01$ with respect to the reference group. (Student's *t*-test).

In addition, significantly lower serum oxytocin values were found in FM patients with a previous CFS diagnosis compared to patients without CFS diagnosis ($p < 0.05$). No significant differences were found in serum levels of oxytocin in FM patients with CFS codiagnosis with respect to the reference group. Nevertheless, FM patients without CFS diagnosis had higher serum levels of oxytocin compared to the reference group ($p < 0.01$).

3.3. Physical Activity/Sedentarism Levels Determined via Accelerometry

Table 4 shows physical activity/sedentarism parameters determined via accelerometry in the total group of patients with FM compared to the reference group. Although no significant differences were found in caloric expenditure (METs), the total group of FM patients showed lower activity bouts ($p < 0.05$) and shorter time of activity bouts, both

total time ($p < 0.05$) and average time ($p < 0.05$), as well as lower step counts ($p < 0.01$) with respect to the reference group. Related to sedentary parameters, total FM patients presented higher number of sedentary bouts (although without significant differences) and longer total sedentary time ($p < 0.01$) with respect to the reference group.

Table 4. Physical activity levels and sedentary lifestyle determined via accelerometry.

	RG	Total FM Patients
Metabolic rate (METs)	1.61 ± 0.09	1.46 ± 0.04
Activity bouts (<1 min)	95.90 ± 13.36	67.27 ± 6.33 [a]
Total Time in Activity bouts (min)	1968.00 ± 334.77	1190.51 ± 135.44 [a]
Average Time per Activity bout (min)	19.33 ± 1.18	16.68 ± 0.73 [a]
Steps count (n° steps)	109,631.00 ± 9800.09	76,069.51 ± 4841.51 [aa]
Sedentary bouts (<1 min)	114.50 ± 8.98	126.96 ± 6.96
Total Time in Sedentary bouts (min)	2567.00 ± 131.44	2902.41 ± 142.54 [a]
Average Time per Sedentary bout (min)	23.07 ± 1.21	23.28 ± 0.66

Data are expressed as mean ± SEM. RG: Reference Group, FM: Fibromyalgia, MET: Metabolic Equivalent of Task. [a] $p < 0.05$, [aa] $p < 0.01$ with respect to reference group. (Student's *t*-test).

3.3.1. Influence of CFS Codiagnosis in FM Patients: Accelerometric Study of Physical Activity

Figure 5 represents physical activity parameters measured via accelerometry in FM patients with and without previous CFS diagnosis, separately. No significant differences were found between FM patients with and without CFS diagnosis in any of the parameters studied: METs (Figure 5a), activity bouts (Figure 5b), total duration of activity bouts (Figure 5c), average duration of activity bouts (Figure 5d) and step count (Figure 5e). However, as expected, both experimental groups showed, in general, lower values of objective physical activity than the reference group; FM patients without CFS codiagnosis presented significantly lower total and average times of activity bouts ($p < 0.05$ in both) and step counts ($p < 0.01$). FM patients with a previous CFS diagnosis presented lower total time and total activity bout counts ($p < 0.05$ in both) and lower step counts ($p < 0.01$).

3.3.2. Influence of CFS Codiagnosis in FM Patients: Accelerometric Study of Sedentarism

Overall, no significant differences were found between FM patients with or without CFS codiagnosis in any of the sedentary parameters determined via accelerometry (Figure 6): sedentary bouts (Figure 6a), total time (Figure 6b) and average time (Figure 6c) of sedentary bouts. Although without significant differences, FM patients with and without CFS codiagnosis showed higher sedentary bouts and total time of sedentary bouts than the reference group.

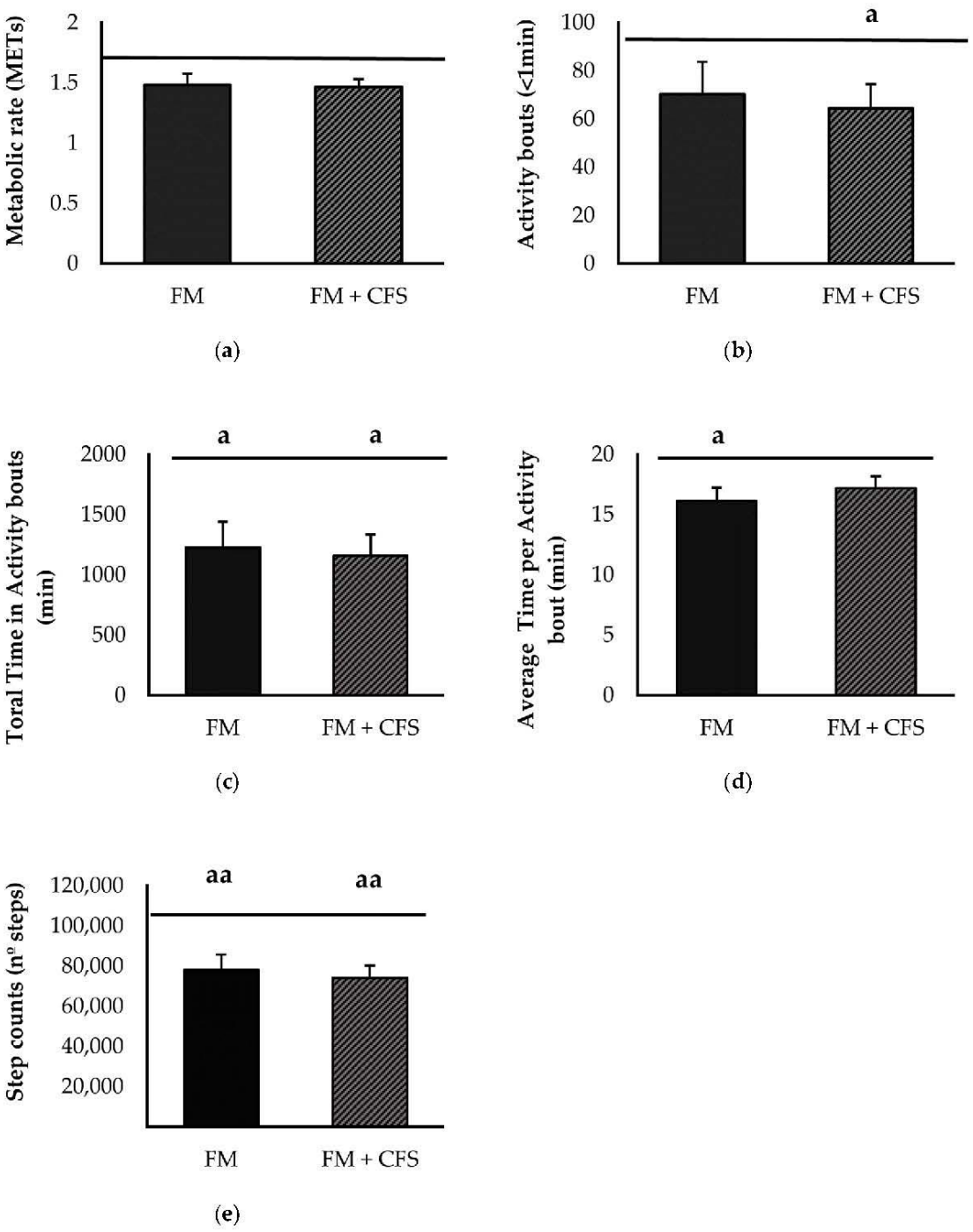

Figure 5. Physical activity levels determined via accelerometry: Metabolic rate (**a**) activity bouts (**b**), total time in activity bouts (**c**), average time per activity bout (**d**) and steps counts (**e**) in FM patients with (FM + CFS, $n = 17$) or without (FM, $n = 17$) CFS diagnosis. The horizontal line represents values obtained in the age-matched reference group of "healthy" women. Columns represent the mean ± SEM of independent assays performed in duplicate for each participant. FM: Fibromyalgia, CFS: Chronic Fatigue Syndrome, MET: Metabolic Equivalent of Task. ^{a} $p < 0.05$, ^{aa} $p < 0.01$ with respect to the reference group. (Student's *t*-test).

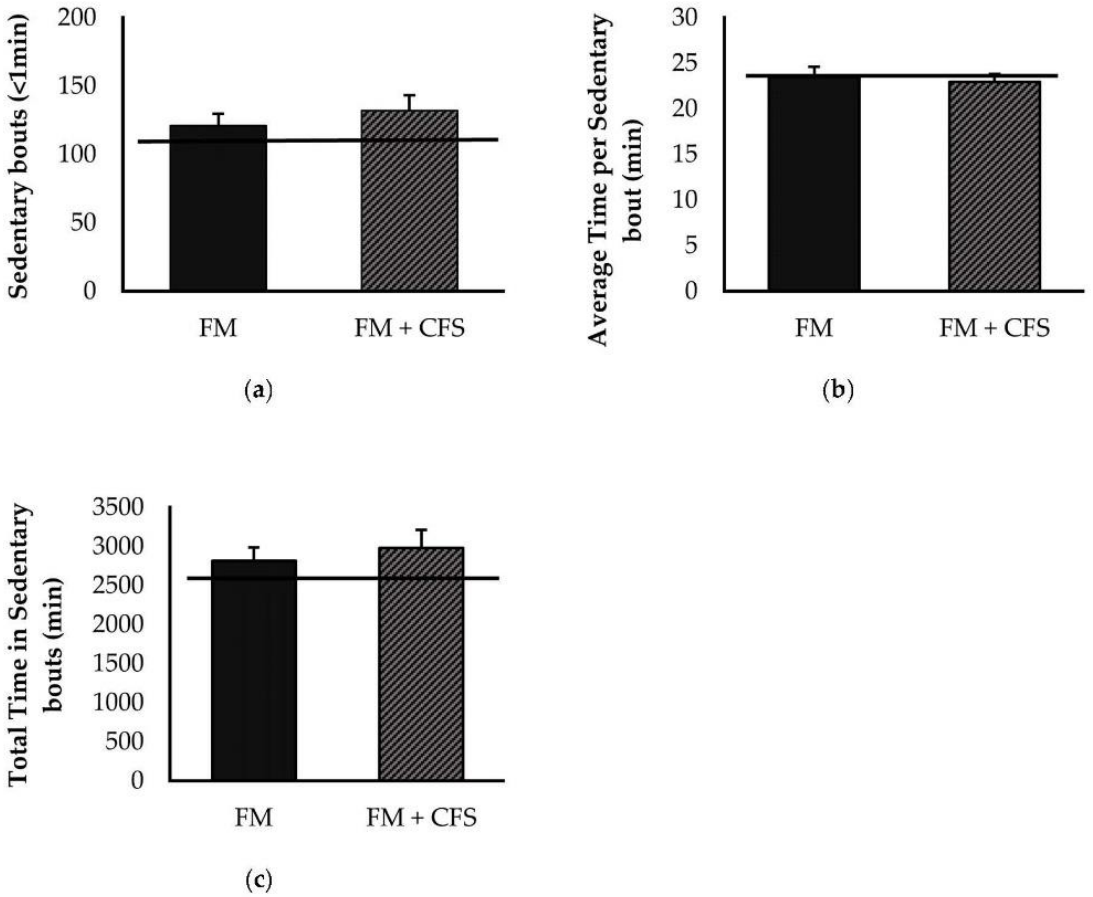

Figure 6. Levels of sedentary lifestyle determined by accelerometry: sedentary bouts (**a**) total time in sedentary bouts (**b**), average time per sedentary bout (**c**) in FM patients with (FM + CFS, $n = 17$) or without (FM, $n = 17$) CFS diagnosis. The horizontal line represents values obtained in the age-matched reference group of "healthy" women. Columns represent the mean ± SEM of independent assays performed in duplicate for each participant. FM: Fibromyalgia, CFS: Chronic Fatigue Syndrome (Mann–Whitney *u*-test).

4. Discussion

In FM syndrome, the terms woman and pain are deeply related; however, it is also characterized by other disturbances such as sensitivity, pressure allodynia, hyperalgesia, sleep disturbances (nocturnal awakenings, intensification of pain after rest, non-restorative sleep), fatigue, psychological disturbances, etc. [16,36,37]. Despite its increasing prevalence, especially in developed countries, its etiopathogenesis remains unclear and its diagnosis remains a challenge [38,39]. Moreover, it is frequently associated with other syndromes such as CFS, comorbid in up to 80% of cases, where the words woman and fatigue go hand in hand, and where many of the clinical manifestations of CFS are similar to those of FM; therefore, those similar pathophysiological mechanisms are assumed in both processes [16,40–43]. However, are they so similar? Is there a possibility that there may sometimes be an overlap in the diagnosis of these two syndromes?

It is conceivable that women with a codiagnosis of these two syndromes might be more affected, in terms of quality of life, pain, stress management, and even physical activity, than those without CFS codiagnosis. Indeed, some authors have suggested differences in the association between cognitive performance and pain, noting that comorbidity of CFS and FM should be considered [44].

However, a very recent study conducted by our research group with more than 70 women, with very uniform lifestyles, showed that CFS codiagnosis did not negatively

affect the psychological state and already impaired quality of life of patients with FM [16]. In a representative group of these patients, we corroborated in the present investigation that the previous CFS diagnosis in women with FM does not worsen the already altered psychological state of these women, who have higher levels of depression, stress, pain, anxiety, and even fear and anxiety towards to COVID-19, compared to an age-matched reference group of healthy women.

In this context, in the present research, we have sought to determine possible differences in the performance of physical activity and psychoneuroimmunoendocrine biomarkers in women with FM, with and without a previous CFS diagnosis. Fatigue is one of the most commonly used criteria for the diagnosis of FM, and of course, since it is its main symptom, also of CFS. Although Fukuda and coworkers [2] recommend specifically assessing the presence and characteristics of fatigue and other associated symptoms in FM, it is still a highly subjective criterion and is closely associated with other types of previous organic or mental illnesses that cause fatigue or morbid obesity. Because of this fatigue, women with FM tend to be less physically active than healthy people [16,45]. This is equally or more so in the case of CFS, where fatigue becomes the biggest obstacle for these women, not only in physical activity, but also in activities of daily living. The problem lies in the fact that most studies use self-reports or questionnaires to measure activity levels in patients with FM, and although this is a quick and easy method, it is subject to response bias [46,47], as these patients suffer from impaired cognitive function [48]; therefore, self-reported physical activity frequently differs from objectively measured physical activity (e.g., via accelerometry) in patients with FM [49]. In a previous study carried out by our research team, women with FM reported less activity than healthy women, and the CFS codiagnosis did not negatively affect self-reported activity levels despite having reported higher levels of subjective fatigue than women without CFS codiagnosis [16]. However, as we have discussed, subjective measurement of fatigue cannot serve as a substitute for objective monitoring measured with an accelerometer, although it may provide additional information on perceived activity [50]. To objectify levels of physical activity and fatigue, in the present investigation, we have also used the technique of accelerometry, which is very novel in the differential study of FM and CFS, as there are no published studies using this technique to determine levels of physical activity in these two syndromes comparatively. As expected, in the present investigation, we observed that patients with FM have worse levels of physical activity and higher levels of sedentary lifestyle than healthy people; however, we did not find a clear influence of previous CFS diagnosis, even though the latter group again reported higher levels of fatigue. Since objective fatigue was not found to be a differentiating symptom of these two pathologies, we decided to target levels of neuroimmunoendocrine mediators (of inflammation and stress) as potential inducers of objective and perceived pain in both FM patients without previous CFS diagnosis and those with CFS codiagnosis.

The relationship between pain and stress has long been well-known. A lack of control over cortisol suppression after acute stress has been demonstrated in FM, an abnormality that has also been found in individuals with psychiatric disorders [51]. Overall cortisol levels as well as release peaks in individuals with FM are higher than in healthy individuals and individuals with rheumatoid arthritis and, therefore, an altered functioning of HPA axis occurs [3,36,52,53], with this alteration being involved in a multitude of disorders, as proposed by most psychobiotic theories [51]. However, it has been shown in other studies that morning serum [54], saliva [55,56], or hair [56] levels of morning cortisol are lower in individuals with chronic musculoskeletal pain [57], or CFS [54]. Klimas and co-workers [11] propose a loss in the fine regulation of the fight–flight mechanism in CFS, being delayed and attenuated in its amplitude. This hypofunction of the HPA axis in CFS and the lower basal cortisol concentration in these patients has been linked to levels of perceived fatigue [58,59]. Our results are in line with those described by these investigations, as we found that the total group of FM patients has higher cortisol levels than the reference group. However, when we separate the women with a previous CFS

diagnosis, we observe that this group has lower cortisol levels than the patients diagnosed only with FM, approaching the values of our reference group of healthy women. It could be hypothesized that the "hyperfunction" of the HPA axis due to FM could be compensated by the hypofunction in CFS. Dysregulation of the HPA axis has also been described by a poor release of DHEA, another endogenous steroid hormone released by the adrenal glands, which may play an etiological role in the maintenance of FM symptomatology [60], as it modulates inflammatory responses through direct inhibition of IL-6 and TNF-α activity [61] and indirectly through promotion of IL-10 [62]. Indeed, decreasing DHEA levels with age have been linked to the development of FM symptomatology throughout life [63]. However, in our results, we found no significant differences in DHEA concentration with respect to the reference group, and no differences between female FM patients with and without a previous CFS diagnosis. Other authors found similar results, proposing that low DHEA levels are more related to age or a postmenopausal state than to the disease itself [60].

Dysregulation of the bidirectional interaction of the cytokine-HPA axis can aggravate inflammatory conditions, and it underlies most autoimmune and inflammatory pathologies, due to a reduced response of the HPA axis to cytokines or to the development of glucocorticoid resistance [64,65]. Thus, disruption of this feedback in FM is associated with a severely dysregulated interaction between the immune/inflammatory and stress responses, particularly mediated by systemic IL-8 and cortisol, but also by other inflammatory cytokines released by monocytes and other stress mediators, such as noradrenaline [3,4,6,66]. In this context, the HPA axis failed to control the increase in pro-inflammatory cytokines [4,5]. Interestingly, the results obtained in the present investigation show this dysregulation in the interaction between the IL-8-mediated inflammatory response and the cortisol-mediated stress response only in patients without a previous CFS diagnosis. These results would suggest that patients with a CFS diagnosis may be overdiagnosed with FM through subjective questionnaires and without the analysis of objective biomarkers, since all previous investigations of our research group showed that women with FM showed elevated levels of IL-8 in relation to healthy women [3]. Our results would not support the idea of Russel and co-workers, who suggested that cytokine levels of IL-1α, IL-6 and IL-8 may serve as robust biomarkers also in the detection of CFS [67].

Additionally, related to the higher levels of pain and depression reported by these patients, we measured noradrenaline and serotonin as objective biomarkers. Indeed, some research has reported that noradrenaline and serotonin reuptake inhibitors are beneficial pharmacological treatments in FM patients [68], probably due to their influence also on the inflammatory response. However, other recent studies have highlighted very poor or no effects [69,70]. The lower systemic serotonin levels in FM patients, already reported in other studies [4,71], and which could explain the lower pain threshold of these patients [72], are clearly consistent with the higher levels of depression perceived by FM patients, both without and with CFS codiagnosis. However, as with perceived levels of fatigue and physical activity, and with neuroimmunoendocrine dysregulation as the mechanism underlying pain, patients with a previous CFS diagnosis showed higher systemic serotonin concentrations than FM patients without CFS, with values very close to those of the control group of healthy women. Again, these results seem to suggest that the CFS diagnosis may induce overdiagnosis of FM through perceived symptoms, which cannot, however, be corroborated using objective biomarkers. This is also true for elevated oxytocin and noradrenaline levels, which are only found to be elevated in FM patients without a CFS codiagnosis. In line with the results obtained for noradrenaline levels, many previous studies reflect higher noradrenaline concentrations in FM patients compared to healthy women [3,4], even without being accompanied by elevated adrenaline levels [73]. Although noradrenaline in healthy conditions can inhibit the release of inflammatory cytokines by immune cells, it has been described that in inflammatory pathologies, it could induce their release [74], a fact that has also been indicated in FM patients [4] and which also explains the results of the present research in the context of neuroimmunoendocrine dysregulation in this disease.

In conclusion, we can say, firstly, that the deterioration in perceived health in patients with FM is corroborated by deterioration in objectively determined physical activity parameters and biomarkers of inflammation and stress. In turn, our results indicate that while perceived health related to fatigue and physical activity capacity, psychological disorders and pain are not affected by a previous CFS diagnosis; physical activity and sedentary lifestyle parameters and objective neuroimmunoendocrine biomarkers related to stress, depression and pain were only manifested in patients without a CFS diagnosis. This suggests a possible overdiagnosis of FM in CFS patients when it is assessed only through perceived symptoms and not with objective immunophysiological parameters. Nevertheless, although the present results can have clinical significance per se, further studies comparing symptoms and objective biomarkers with a big cohort of CFS patients are proposed in order to avoid overdiagnosis of FM.

Author Contributions: Conceptualization, E.O. (Eduardo Ortega) and M.D.H.; methodology, E.O. (Eduardo Otero), M.D.H. and I.G.; formal analysis, E.O. (Eduardo Otero); investigation, M.D.H., E.O. (Eduardo Ortega), I.G. and E.O. (Eduardo Otero); writing—original draft preparation, M.D.H. and E.O. (Eduardo Otero); writing—review and editing, M.D.H., I.G. and E.O. (Eduardo Ortega); supervision, E.O. (Eduardo Ortega); project administration, E.O. (Eduardo Ortega); funding acquisition, E.O. (Eduardo Ortega). All authors have read and agreed to the published version of the manuscript.

Funding: This research was partially funded by the Gobierno de Extremadura—Fondo Europeo de Desarrollo Regional, Spain (GR21079) and by aid to promote the hiring of research support staff in the Autonomous Community of Extremadura (TE-0027-19).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Bioethics Committee of University of Extremadura, Spain (N Reg. 73/2021, 9 June 2021). The study was registered on ClinicalTrials.gov (identifier: NCT05323838).

Informed Consent Statement: Written informed consent was requested from all participants prior to participation in the study.

Data Availability Statement: The raw data supporting the conclusions of the manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

Acknowledgments: We are grateful to the associations of Fibromyalgia and Chronic Fatigue Syndrome belonging to the community of Extremadura: EXISTIMOS (Badajoz, Spain).

Conflicts of Interest: The authors declare no conflict of interest.

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Publicación 3.

Hinchado, M. D., Quero-Calero, C. D*., Otero, E., Gálvez, I., y Ortega, E. (2023). Synbiotic Supplementation Improves Quality of Life and Inmunoneuroendocrine Response in Patients with Fibromyalgia: Influence of Codiagnosis with Chronic Fatigue Syndrome. *Nutrients,* 15(7), 1591. https://doi.org/10.3390/nu1507159

Título: *"La Suplementación con un Simbiótico Mejora la Calidad de Vida y la Respuesta Inmunoneuroendocrina en Pacientes con Fibromialgia: Influencia del Codiagnóstico con Síndrome de Fatiga Crónica"*

Resumen

La fibromialgia (FM) y el síndrome de fatiga crónica (SFC) son dos afecciones médicas en cuyo diagnóstico predominan el dolor, la fatiga, la desregulación inmunitaria/inflamatoria, así como diversos trastornos de la salud mental, sin que exista evidencia de un consenso claro sobre el tratamiento de la FM y el SFC. El objetivo principal de esta investigación fue analizar los posibles efectos de un simbiótico (Synbiotic, Gasteel Plus® (Heel España S.A.U.), mediante el estudio de citocinas proinflamatorias/antiinflamatorias (IL-8/IL-10) y biomarcadores neuroendocrinos (cortisol y DHEA), con el fin de evaluar la interacción entre las respuestas inflamatorias y de estrés mediadas por el eje citocinas-HHA (Hipotálamo-Hipófisis-Adrenal), así como la salud mental y física mediante análisis de composición corporal, acelerometría y cuestionarios previamente validados. Las participantes eran mujeres diagnosticadas con FM con o sin diagnóstico de SFC. Cada participante fue evaluada al inicio y después de la intervención, que duró un mes. La intervención con simbióticos redujo los niveles de estrés percibido, ansiedad y depresión, y mejoró la calidad de vida durante las actividades cotidianas. Además, el simbiótico generó una activación del eje HHA (liberación fisiológica de cortisol) que puede compensar el aumento del estado inflamatorio (IL-8 elevada) observado al inicio en las pacientes con FM. No se produjeron cambios perjudiciales en la composición corporal ni en los parámetros del sueño, así como en la mayoría de los parámetros relacionados con la actividad física/sedentarismo estudiados mediante acelerometría. Se concluye que los suplementos nutricionales simbióticos pueden mejorar la interacción inmunoneuroendocrina desregulada que implica respuestas inflamatorias y de estrés en mujeres diagnosticadas de FM, particularmente en aquellas sin diagnóstico previo de SFC; así como su percepción de los niveles de estrés, ansiedad, depresión y calidad de vida.

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Synbiotic Supplementation Improves Quality of Life and Inmunoneuroendocrine Response in Patients with Fibromyalgia: Influence of Codiagnosis with Chronic Fatigue Syndrome

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Abstract: Fibromyalgia (FM) and chronic fatigue syndrome (CFS) are two medical conditions in which pain, fatigue, immune/inflammatory dysregulation, as well as various mental health disorders predominate in the diagnosis, without evidence of a clear consensus on the treatment of FM and CFS. The main aim of this research was to analyse the possible effects of a synbiotic (Synbiotic, Gasteel Plus® (Heel España S.A.U.), through the study of pro-inflammatory/anti-inflammatory cytokines (IL-8/IL-10) and neuroendocrine biomarkers (cortisol and DHEA), in order to evaluate the interaction between inflammatory and stress responses mediated by the cytokine-HPA (hypothalamic-pituitary-adrenal) axis, as well as mental and physical health using body composition analysis, accelerometry and previously validated questionnaires. The participants were women diagnosed with FM with or without a diagnostic of CFS. Each participant was evaluated at baseline and after the intervention, which lasted one month. Synbiotic intervention decreased levels of perceived stress, anxiety and depression, as well as improved quality of life during daily activities. In addition, the synbiotic generated an activation of HPA axis (physiological cortisol release) that can compensate the increased inflammatory status (elevated IL-8) observed at baseline in FM patients. There were no detrimental changes in body composition or sleep parameters, as well as in the most of the activity/sedentarism-related parameters studied by accelerometry. It is concluded that synbiotic nutritional supplements can improve the dysregulated immunoneuroendocrine interaction involving inflammatory and stress responses in women diagnosed with FM, particularly in those without a previous CFS diagnostic; as well as their perceived of levels stress, anxiety, depression and quality of life.

Keywords: fibromyalgia; chronic fatigue syndrome; synbiotic; inflammation; stress; IL-8; IL-10; cortisol; dehydroepiandrosterone

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Citation: Hinchado, M.D.; Quero-Calero, C.D.; Otero, E.; Gálvez, I.; Ortega, E. Synbiotic Supplementation Improves Quality of Life and Inmunoneuroendocrine Response in Patients with Fibromyalgia: Influence of Codiagnosis with Chronic Fatigue Syndrome. *Nutrients* **2023**, *15*, 1591. https://doi.org/10.3390/nu15071591

Academic Editor: Herbert Ryan Marini

Received: 28 February 2023
Revised: 16 March 2023
Accepted: 23 March 2023
Published: 25 March 2023

1. Introduction

Fibromyalgia (FM) is a common chronic pain condition, in which patients may also experience a variety of other symptoms, including sleep disturbances, fatigue, stiffness, frequent episodes of pain and mental health problems, as well as possible gastrointestinal disorders [1]. In addition, and according to the American College of Rheumatology [2],

such a generalised non-joint pain state occurs for at least three months in duration, predominantly in women over 50 years of age. Chronic fatigue syndrome (CFS) is a condition characterized by persistent and debilitating fatigue lasting at least six months [3]. CFS is the most common comorbidity in patients with FM, ranging from 20–81% [4].

The origin of FM and CFS is unknown, although alterations in the central nervous system, as well as abnormalities in muscle physiology and immune/inflammatory response are suggested as the main causes [5–7]. Increasing evidence suggests that the gut microbiota of people suffering from FM and CFS differs from that of healthy individuals, with several studies showing lower values of *Escherichia coli* and *Bifidobacterium* and significantly higher numbers of enterococci compared to healthy controls, potentially leading to various gastrointestinal disorders [8,9]. In addition, several inflammatory and stress biomarkers have been found in our laboratories in previous studies (increased release of pro-inflammatory cytokines including IL-8 together with greater levels of cortisol and noradrenaline in the blood) when comparing FM patients with healthy individuals [6,7,10].

In this context, several food supplements are currently proposed for the improvement of symptoms in FM and CFS, among which we can highlight probiotics [11–14], as well as synbiotics [15], the latter being very scarce in the literature. Probiotic therapy, according to other authors [16], may change the gut microbiota, enhance mucosal barrier function, reduce pro-inflammatory cytokines and probably have a favourable effect on mood in people who have emotional symptoms and elevated inflammatory immune signals, as well as improvements in cognitive symptoms through neuroimmunoendocrine enhancements [12].

Therefore, the main objective of this research was to analyse the possible effects of a synbiotic in people diagnosed with fibromyalgia through the study of various objective immune/inflammatory and stress biomarkers, as well as perceived mental and physical health parameters, and objective levels of physical activity determined by accelerometry. The existence of a previous diagnosis of chronic fatigue syndrome was also considered to influence some of the parameters evaluated. To the best of our knowledge, this is the first investigation in this context, all this with the aim of helping to a correct prescription of adjuvant treatments that improve the quality of life of these patients, allowing a better differential diagnosis between both syndromes.

2. Materials and Methods

2.1. The Synbiotic

Within the probiotic strains present in Gasteel Plus® (Heel España S.A.U. laboratories, Madrid, Spain) are *Bifidobacterium lactis* CBP-001010, *Lactobacillus rhamnosus* CNCM I-4036, and *Bifidobacterium longum* ES1, as well as fructooligosaccharides (200 mg) as a prebiotic. Each Gasteel Plus® bar (300 mg) contains 1×10^9 colony forming units (CFU) of freeze-dried powdered bacteria in addition to 1.5 mg zinc, 8.25 g selenium, 0.75 g vitamin D and maltodextrin as an excipient.

2.2. Participants

A total of 15 women, diagnosed with FM, with ($n = 7$) or without ($n = 8$) previously diagnosed with CFS. Inclusion criteria included: (i) women between 40–67 years of age, (ii) diagnosed with FM (according to ACR diagnostic criteria) [2], with or without CFS (according to Fukuda and co-workers criteria) [3] by a specialist rheumatologist or internal medicine physician. Participants were excluded if they: (i) consumed any type of probiotic food supplement, (ii) were taking antibiotics, corticosteroids or anti-cytokines therapy during the treatment period, (iii) had a positive medical diagnosis of depression or (iv) were periodically performing scheduled therapeutic physical activity in the two months prior to the accelerometry tests. All participants belonged to a FM association EXISTIMOS ® in Badajoz, Extremadura (Spain).

Descriptive data of the participants are shown in Table 1. All participants are Caucasian women and have been diagnosed with FM for more than two years (with or without a

previous codiagnosis of CFS). No significant differences were found in either age or BMI between the two experimental groups.

Table 1. Descriptive data of the participants.

Variable	Total FM Patients (n = 15)	FM (n = 8)	FM + CFS (n = 7)
Gender (%)	Women (100%)	Women (100%)	Women (100%)
Ethnic group (%)	Caucasian (100%)	Caucasian (100%)	Caucasian (100%)
Duration of FM/CFS diagnosed (years)	>2	>2	>2
Age (years)	59.38 ± 2.35	63.00 ± 3.35	55.75 ± 3.18
BMI (kg/m^2)	29.19 ± 1.50	29.78 ± 1.35	28.60 ± 2.82

The data are represented as mean ± SEM. BMI: Body Mass Index; CFS: Chronic Fatigue Syndrome; FM: Fibromyalgia.

2.3. Procedures

This experimental research is part of a pilot study with the aim of identifying the potential benefits of the synbiotic Gasteel Plus® (Heel España S.A.U.) in women diagnosed with FM, with or without a previous diagnostic of CFS. To this end, the subjects were required to maintain a diet similar to the one prior to the treatment period (control diary) and to maintain it during the 30 days of taking the synbiotic. Participants signed an informed consent form prior to the protocol, which was approved in advance by the University of Extremadura committee in accordance with the Council of Europe Directives and the Declaration of Helsinki (registration number 13/2020).

The measurements were performed on two days (baseline and post-test) with a 30-day separation between them in which the participants had to consume the synbiotic, taking one stick a day, preferably in the morning and mixed in water. The order of the tests, the materials used and the members of the research team were the same for the pre-test and post-test so as not to interfere with any of the procedures. Accelerometers and questionnaires were distributed one week before the pre-test and post-test and collected the day of the blood sampling and test determinations.

2.4. Biompedance Analysis: Determination of Body Composition Measurements

Body composition was analysed using the BIA TANITA DC-360 digital scale (Tanita, Tokio, Japan), with measurement frequencies between 6.25 kHz/50 kHz. The following data were obtained: weight (kg), % fat mass, body water, muscle mass (kg), bone mass and visceral fat level. BMI was calculated using the formula weight/height^2 expressed in kg/m^2. All participants were measured barefoot, lightly clothed and fasting.

2.5. Accelerometry: Determination of Objective Levels of Physical Activity, Sedentary Lifestyle and Sleep Quality

The Actigraph wGT3X-BT, a compact and lightweight 3-axis accelerometer (4.6 × 3.3 × 1.5 cm, 19 g) with a response rate of 30–100 Hz, was the accelerometer used in this study. Several objective indicators, including physical activity and intensity, energy expenditure, metabolic equivalent (METs), number of steps taken per week, amount of time spent sitting still, exercise, latency and sleep efficiency are measured by the device. Except at occasions when the accelerometer's regular operation might be impacted, participants wore an accelerometer fastened to an elastic band on their non-dominant wrist for 7 days in a row (shower or water-related activity). Actilife 6 was used specifically for the analysis of the files the accelerometer produced (ActiGraph, LLC., Pensacola, FL, USA).

2.6. Questionnaires: Determination of Perceived Levels of Stress, Anxiety, Fatigue, Pain, Depression, Sleep Quality and Quality of Life

In order to determine the perceived levels of mental health and quality of life of the participants, several scientifically validated questionnaires were used, among them:

- The Spanish version [17] of the Beck Depression Inventory (BDI) was used to determine possible signs of depression in the past week. Higher scores indicate higher levels of depression.
- State-Trait Anxiety Inventory (STAI), to analyse the levels of anxiety presented at a specific time and in general. A Spanish version [18] was used for this purpose. Higher scores indicate higher levels of anxiety.
- The Perceived Stress Scale (PSS), to assess the frequency with which participants experience stressful situations and thoughts in the last month. Higher scores indicate higher levels of stress. Remor was used in its Spanish version [19].
- Brief Pain Inventory (BPI). Used to determine the intensity and interference of pain in daily activities. The greater the perception of pain, the higher the score obtained. A Spanish version [20] was used.
- Brief Fatigue Inventory (BFI). This questionnaire measures the intensity of fatigue in the last 24 h and its interference with daily activities and work. The higher the perception of fatigue, the higher the score obtained [21].
- Healthy Lifestyle and Personal Control Questionnaire (HLPCQ). The Healthy Lifestyle and Personal Control Questionnaire is composed of several sections referring to type of diet, organised physical exercise, as well as social and mental balance [22].
- Pittsburgh Sleep Quality Questionnaire (PSQI). This questionnaire analyses various parameters related to subjective sleep quality: latency, duration, efficiency and disturbances, as well as consumption of sleeping pills. The Spanish version of the questionnaire was used [23].
- FIQ (Fibromyalgia Impact Questionnaire). A Spanish version [24] was used to assess the impact of FM on physical and mental functions (pain, tiredness, fatigue, stiffness, anxiety and depression). Higher scores indicate a worse health condition.
- Gastrointestinal Health Questionnaire. This questionnaire provides insight into gastrointestinal function in adults by identifying the level of severity of gastrointestinal symptoms. The higher the final score, the more severe the symptoms [25].
- COVID-19 questionnaires:
- CAS (Coronavirus Anxiety Scale). The higher the score, the greater the sense of anxiety. Higher scores are related to higher anxiety towards COVID-19 [26].
- FCV-19S (Fear of Coronavirus). The higher the score, the greater the sense of fear of the coronavirus [27].

2.7. Blood Sampling: Determination of Inflammatory and Stress Biomarkers

Blood of fasting individuals was extracted at 8 a.m. and placed in collection tubes with EDTA anticoagulant and coagulation agents to separate plasma and serum, respectively. Both the plasma and the serum were centrifuged for 10 min at 1600 and 1800× *g*, respectively. After serum and plasma samples were collected, they were coded and gradually cooled at −20 °C.

For the determination of the pro- and anti-inflammatory cytokines studied (IL-8 and IL-10), the competitive inhibition enzyme-linked immunoassay (ELISA) technique was used using the Human IL-8 and IL-10 Kits (Diaclone Biotech, Besancon, France), respectively. Stress hormones such as cortisol (DetectX® Cortisol enzyme immunoassay kit, Arbor Assays Inc., Ann Arbor, MI, USA) and the hormone dehydroepiandrosterone, DHEA (DEH3344; Demeditec Diagnostics GmbH, Kiel, Germany) were also analysed by ELISA.

2.8. Statistics

The statistical analysis was conducted using IBM statistics SPSS v20.0 program. The Shapiro–Wilk test was employed to verify the normality of the data. Student *t*-test paired

and unpaired were applied to determine how the intervention affected the outcome. The values were given as mean standard error of mean and the significance threshold was taken into account when $p < 0.05$.

3. Results

3.1. Effects of the Synbiotic on Body Composition Measurements Determined by Bioelectrical Impedance Analysis (BIA)

The results in Table 2 show that there were no significant changes in weight, body fat mass percentage, bone mass, total body water percentage or muscle mass after consumption of the synbiotic, suggesting that the participants maintained the same diet during the study protocol and that it did not produce any detrimental effect on the body composition of the subjects. Only the visceral fat index decreased statistically significant ($p < 0.05$) in the group of FM patients without a codiagnosis of CFS, but without a physiological relevance.

Table 2. Results of body composition measurements determined by Bioelectrical Impedance Analysis (BIA).

	Total FM Patients (*n* = 15)		**FM (*n* = 8)**		**FM + CFS (*n* = 7)**	
	Basal	**Post**	**Basal**	**Post**	**Basal**	**Post**
Weight (kg)	76.93 ± 3.76	76.72 ± 3.76	75.33 ± 2.47	74.56 ± 2.47	78.76 ± 7.91	79.19 ± 7.62
Body fat mass (%)	40.15 ± 1.55	40.43 ± 1.54	41.25 ± 1.55	41.50 ± 1.53	39.05 ± 2.80	39.36 ± 2.79
Bone mass (kg)	2.30 ± 0.05	2.30 ± 0.06	2.33 ± 0.03	2.30 ± 0.05	2.28 ± 0.11	2.30 ± 0.11
Body water (%)	41.25 ± 1.03	41.34 ± 1.05	40.29 ± 1.42	40.29 ± 1.44	42.21 ± 1.59	42.40 ± 1.62
Muscle mass (kg)	42.78 ± 1.09	42.83 ± 1.07	42.99 ± 0.87	42.61 ± 1.08	42.56 ± 2.10	43.05 ± 1.98
Visceral fat index	9.53 ± 0.87	9.56 ± 0.75	10.31 ± 0.80	10.13 ± 0.81 *	8.75 ± 1.45	9.00 ± 1.33

* $p < 0.05$ indicate statistically significant difference with respect to the BASAL values. The data are represented as mean ± SEM. CFS: Chronic fatigue Syndrome; FM: Fibromyalgia.

3.2. Effects of the Synbiotic on Physical Activity/Sedentarism Levels and Sleep Quality Determined by Accelerometry

In general, the synbiotic administration did not induce changes in the objective determination of activity/sedentarism and sleep parameters evaluated by accelerometry (Table 3). Only a statistically significant decrease was found in the Total Time in Activity bouts in the total FM patients. This may have been due to the fact that during the month under study, temperatures were quite high and may have reduced the normal physical activity of the subjects.

Table 3. Objective results of physical activity, sedentary levels and sleep quality determined by Accelerometry.

	Total FM Patients (*n* = 15)		**FM (*n* = 8)**		**FM + CFS (*n* = 7)**	
	Basal	**Post**	**Basal**	**Post**	**Basal**	**Post**
METs (mL O_2/kg·min)	1.43 ± 0.04	1.41 ± 0.04	1.47 ± 0.06	1.44 ± 0.05	1.37 ± 0.04	1.36 ± 0.05
Activity bouts (<1 min)	58.04 ± 7.59	49.10 ± 7.19	58.71 ± 11.08	54.70 ± 10.62	58.25 ± 9.81	39.25 ± 6.22
Total Time in Activity bouts (min)	974 ± 166.38	759.36 ± 123.17 *	1004.71 ± 251.04	847.86 ± 179.17	921.75 ± 178.188	604.5 ± 118.98
Average Time per Activity bout (min)	15.55 ± 0.98	14.87 ± 0.62	16.05 ± 1.53	15.00 ± 0.74	14.84 ± 1.12	14.68 ± 1.19
Sedentary bouts (<1 min)	125.01 ± 7.95	120.67 ± 8.17	124.28 ± 12.58	119.14 ± 11.02	126.01 ± 9.19	122.8 ± 13.57
Total Time in Sedentary bouts (min)	2885.67 ± 235.55	2599.91 ± 155.15	2860.85 ± 335.14	2561.57 ± 219.28	2920.41 ± 360.31	2653.61 ± 238.06
Average Time per Sedentary bout (min)	23.22 ± 1.43	21.84 ± 0.72	22.95 ± 1.02	21.80 ± 0.92	23.61 ± 3.36	21.90 ± 1.23
Sleep latency (min)	0.70 ± 0.14	0.75 ± 0.14	1.14 ± 0.56	1.14 ± 0.86	0.91 ± 0.25	0.72 ± 0.22
Sleep efficiency (%)	87.71 ± 1.37	87.09 ± 1.37	88.72 ± 1.73	87.79 ± 2.03	86.29 ± 2.30	86.1 ± 1.49
WASO (min)	49.18 ± 5.25	53.37 ± 6.21	45.36 ± 6.15	49.95 ± 9.44	54.54 ± 9.50	58.16 ±7.61

* $p < 0.05$ indicate statistically significant difference with respect to the BASAL values. The data are represented as mean ± SEM. CFS: Chronic fatigue Syndrome; FM: Fibromyalgia; METs: Metabolic Equivalent of Task; WASO: Wakefulness After Sleep Onset.

3.3. Effects of the Synbiotic on Perceived Levels of Depression, Stress, Anxiety, Pain, Fatigue, Sleep Quality and Quality of Life Determined by Questionnaires

The results of perceived health measured through previously validated questionnaires are shown in Table 4. Overall, statistically significant improvements were observed in levels of perceived depression, stress, anxiety and fatigue as well as in the fibromyalgia impact on daily activity ($p < 0.05$) in the total group of FM patients. Specifically, depression and stress only statistically improved in FM patients without a codiagnosis of CFS ($p < 0.05$), although anxiety, fatigue and the impact of FM only statistically improved in the FM + CFS group ($p < 0.05$).

Table 4. Results of perceived levels of stress, anxiety, fatigue, pain, depression, sleep quality and quality of life.

	Total FM Patients (n = 15)		**FM (n = 8)**		**FM + CFS (n = 7)**	
	Basal	**Post**	**Basal**	**Post**	**Basal**	**Post**
Healthy Life and Personal Control Score	63.27 ± 3.38	65.40 ± 3.21	66.13 ± 3.42	69.00 ± 3.99	60.00 ± 6.19	61.29 ± 4.99
Beck's Depression Score	18.67 ± 2.77	15.67 ± 2.82 *	18.13 ± 3.30	14.75 ± 3.24 *	19.29 ± 4.86	16.71 ± 5.03
Perceived Stress Score	31.27 ± 3.02	26.87 ± 2.89 *	31.75 ± 3.40	25.75 ± 3.26 *	30.71 ± 5.48	28.14 ± 5.19
Trait-Anxiety Score	33.93 ± 3.74	31.40 ± 3.51 *	34.38 ± 4.98	31.88 ± 4.78	33.43 ± 6.06	30.86 ± 5.59 *
State-Anxiety Score	33.80 ± 4.33	31.53 ± 4.10	34.25 ± 5.23	31.25 ± 5.25	33.29 ± 7.55	31.86 ± 6.89
Brief Pain Inventory Score	6.24 ± 0.46	5.97 ± 0.46	6.57 ± 0.41	6.22 ± 0.37	5.88 ± 0.87	5.68 ± 0.91
Brief Fatigue Inventory Score	7.28 ± 0.40	6.52 ± 0.57 *	6.95 ± 0.51	6.66 ± 0.41	7.65 ± 0.65	6.35 ± 1.18 *
Pittsburgh Sleep Quality Score	12.60 ± 1.02	11.73 ± 0.81	11.13 ± 0.81	10.25 ± 0.41	14.29 ± 1.86	13.43 ± 1.46
Coronavirus Anxiety Score	2.07 ± 0.85	1.47 ± 0.60	2.88 ± 1.44	1.88 ± 0.97	1.14 ± 0.77	1.00 ± 0.69
Fear of COVID-19 Score	13.85 ± 2.06	13.73 ± 1.73	14.33 ± 4.01	15.50 ± 2.77	13.43 ± 2.03	11.71 ± 1.84 *
Fibromyalgia Impact Questionnaire Score	55.37 ± 3.09	50.51 ± 3.31 *	54.55 ± 3.98	51.35 ± 4.01	56.30 ± 5.13	49.56 ± 5.74 *
Gastrointestinal Health Score	9.73 ± 1.39	9.53 ± 1.41	11.13 ± 1.88	10.88 ± 2.29	8.14 ± 2.04	8.00 ± 1.48

* $p < 0.05$ indicate statistically significant difference with respect to the BASAL values. The data are represented as mean ± SEM. CFS: Chronic fatigue syndrome; FM: Fibromyalgia.

Even though without statistical significance, the synbiotic also improved pain, sleep quality and gastrointestinal health of the participants. Furthermore, coronavirus questionnaires show that participants have less fear about contracting the disease in the FM + CFS group.

3.4. Effects of the Synbiotic on Inmunoneuroendocrine Biomarkers

3.4.1. Inflammatory Biomarkers (IL-8 and IL-10)

Figure 1 shows the effect of the synbiotic on inflammatory biomarkers (IL-8 and IL-10) determined by ELISA. After the intervention, FM patients significantly decreased their systemic IL-8 concentration ($p < 0.05$) (Figure 1a). However, we can observe how this significant decrease in IL-8 concentration only occurred in patients without a codiagnosis of CFS ($p < 0.05$) whose baseline levels were above the reference value (>29 pg/mL) of our laboratory in healthy women [6,7] (Figure 1b). Paradoxically, the group with a previous diagnosis of CFS did not present IL-8 levels above the level compatible with healthy individuals.

In addition, the administration of the synbiotic induced an increase ($p < 0.01$) in the anti-inflammatory cytokine IL-10 only in the group of patients without a diagnosis of CFS (Figure 1d). This effect did not occur in the group of FM patients with a previous diagnosis of CFS, which prevented the determination of statistically significant differences in the total group of patients (Figure 1c).

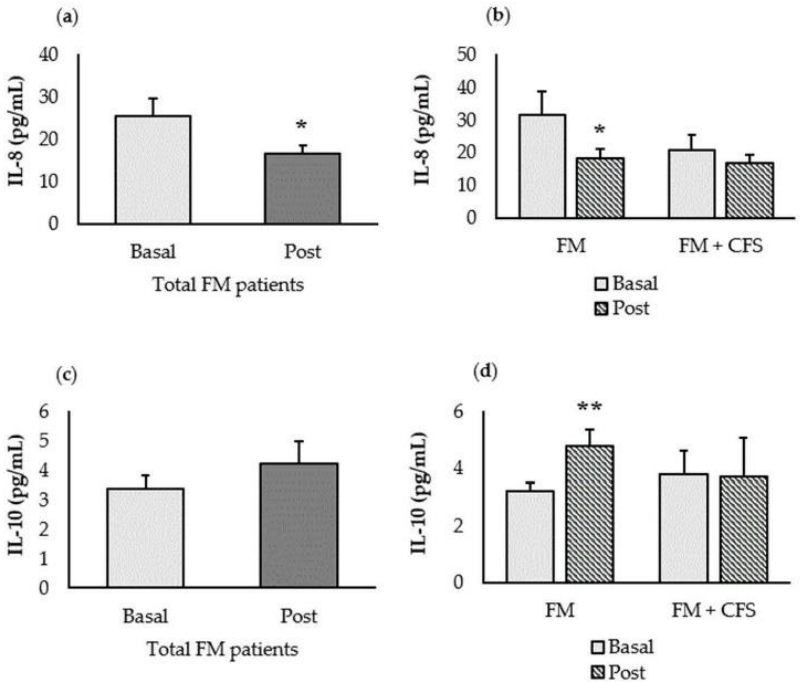

Figure 1. Effect of the synbiotic administration on inflammatory biomarkers. (**a**) IL-8 concentration in the total group of FM patients ($n = 15$), (**b**) IL-8 concentration in the FM patients with (FM + CFS, $n = 7$) or without (FM, $n = 8$) CFS evaluated separately, (**c**) IL-10 concentration in the total group of FM patients ($n = 15$), (**d**) IL-10 concentration in the FM patients with (FM + CFS, $n = 7$) or without (FM, $n = 8$) CFS diagnosed separately. Each column represents the mean ± SEM of the cytokine determination in each patient. * $p < 0.05$ and ** $p < 0.01$ with respect to the baseline.

3.4.2. Stress-Related Biomarkers (Cortisol and DHEA)

The data obtained for the stress-related hormones, cortisol hormone and dehydroepiandrosterone (DHEA), as well as their ratios are shown in Figure 2. A significant increase ($p < 0.05$) in cortisol is observed after the intervention in the entire group of FM patients (Figure 2a), but this increase ($p < 0.05$), together a decrease ($p < 0.01$) in DHEA concentration, was only found in FM patients without a CFS codiagnosis when evaluated separately (Figure 2b).

As a consequence, after synbiotic administration, the Cortisol/DHEA ratio increased significantly both in the total group of patients with FM ($p < 0.05$) (Figure 2e) and in the group of patients with FM without previous CFS diagnosis ($p < 0.01$).

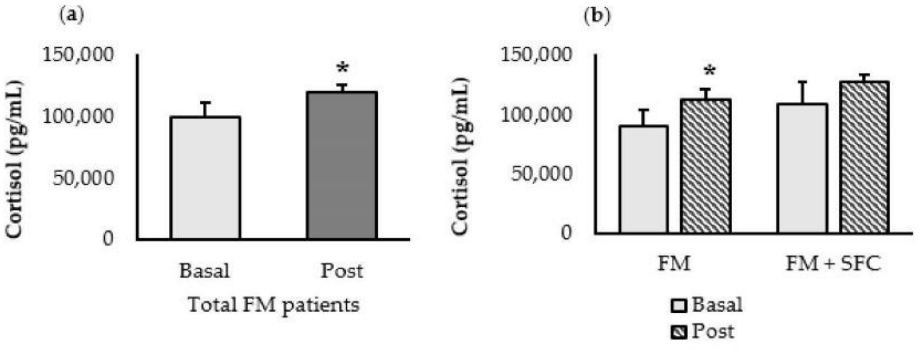

Figure 2. *Cont.*

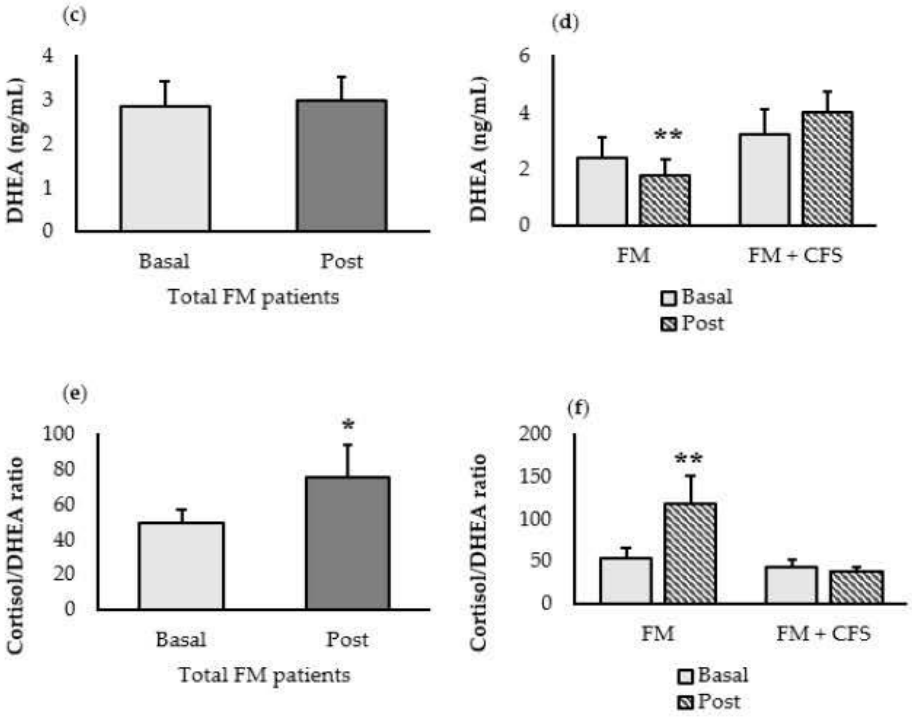

Figure 2. Effect of the synbiotic administration on stress-related biomarkers. (**a**) Cortisol concentration in the total group of FM patients ($n = 15$), (**b**) cortisol concentration in the FM patients with (FM + CFS, $n = 7$) or without (FM, $n = 8$) CFS evaluated separately, (**c**) DHEA concentration in the total group of FM patients ($n = 15$), (**d**) DHEA concentration in the FM patients with (FM + CFS, $n = 7$) or without (FM, $n = 8$) CFS evaluated separately. (**e**) Cortisol/DHEA ratio in the total group of FM patients ($n = 15$), (**f**) Cortisol/DHEA ratio in the FM patients with (FM + CFS, $n = 7$) or without (FM, $n = 8$) CFS evaluated separately. Each column represents the mean ± SEM of the stress-related biomarkers determination in each patient. * $p < 0.05$ and ** $p < 0.01$ with respect to the baseline.

4. Discussion

FM and CFS are two conditions of diffuse aetiology, often codiagnosed and still poorly differentiated nowadays. Although the preferred symptom in FM is pain and in CFS fatigue, they have many others in common. In fact, studies in our laboratory clearly reveal that, FM patients (with or without a previous diagnostic of CFS) present worse perceived levels of stress, anxiety, fatigue, pain, depression, sleep quality and quality life [28].

Moreover, in recent years, the role of the gut microbiome in host health and disease has been increasingly considered and the so-called gut–brain axis is now clearly accepted. Although patients with FM and CFS frequently have gastrointestinal problems, gut dysbiosis has been described as a consequence rather than a cause of fibromyalgia, with some strains of bacteria overgrowing in the small intestine and, in addition, there seems to be a specific profile of some species in people with FM [29] and with CFS [30]. This alteration of the microbiota is associated with many of the symptoms of FM and CFS, such as chronic widespread musculoskeletal pain [31], fatigue, mood and other symptoms [32]. In addition, after anti-microbial treatments, there seems to be a clinical improvement [33].

Although there are no specific treatments for FM patients, some of the most commonly used strategies to improve the health and quality of life of these patients is through exercise [6,34] and nutrition [13]. Nutrition strategies can acquire particular importance in patients with low adherence to exercise programs due to their difficulties to perform physical activities in long programs. Some supplements studied have included vitamin D [35], iron [36] and magnesium [37]. In addition, as we have stated in previous research, particularly in young sedentary and sports people [38,39], probiotics, prebiotics and synbiotics could be beneficial for health, having positive repercussions on gastrointestinal health,

immune and inflammatory response, as well as in other mental health parameters. However, there is little research on the use of probiotics in people diagnosed with FM and/or CFS [12,14,40–43] and even less after consumption of synbiotics. Thus, very interesting are the results of the present investigation showing that after ingesting a synbiotic for a period of 30 days, FM patients improved their mood, stress and anxiety levels, as well as the impact of the disease on their daily activities subjectively determined by validated questionnaires. These findings could be consistent with other research [44,45] which concludes that by increasing intestinal microbial balance, probiotics improve host health and they may also benefit cognitive and psychological functions via the gut–brain axis [11].

In this context, there is also scientific evidence linking FM and CFS to alterations in the central nervous system [46], which could also be a cause or consequence of the intestinal dysbiosis recently described in patients with FM [32]. Furthermore, FM and CFS are associated with a severely dysregulated immune/inflammatory system, as well as a dysregulation of the HPA (hypothalamic-pituitary-adrenal) axis [6,7,10,34], which may, among others, alter the physical and cognitive health of such individuals [12,28], as previously mentioned. This dysregulation may induce an imbalance in pro- and anti-inflammatory cytokines, with a pivotal role in the pathogenesis of FM [47–49]. Probably, an imbalance between pro-inflammatory and anti-inflammatory cytokines leads to chronic peripheral sensitization of the nervous system as a major contributor to pain and the way pain is processed [50,51].

Of these, systemic levels of the pro-inflammatory cytokine IL-8 are the ones frequently seen elevated in rigorously diagnosed FM patients, having even been reported as the best potential biomarker of FM inflammation [6,52–54]. Thus, before intervention, the high levels of pro-inflammatory cytokine IL-8 (more than 29 pg/mL as reference value in our laboratory [6,7] have also been determined in FM patients in the present investigation. After the intervention with the synbiotic, systemic levels of IL-8 decreased in FM patients (to values compatible with those of healthy individuals), together with an increase in the systemic levels of the anti-inflammatory cytokine IL-10, all of this particularly evident in the group of FM patients without a previous diagnostic of CFS.

What is the mechanism used by the synbiotic treatment to regulate the pro-anti-inflammatory cytokine imbalance is the next question that arises. According with the results of the present investigation, some authors [16] proposed that microbiota-induced improve in mucosal barrier function after probiotic administration underly the decrease in pro-inflammatory cytokines that mediate the improvements in emotional and cognitive symptoms via a better inmunoneuroendocrine regulation. In addition, inflammatory and stress responses are bidirectionally regulated. Thus, pro-inflammatory cytokines stimulate the HPA axis, inducing an increase in glucocorticoid levels, which in turn protect the organism from an overproduction of inflammatory cytokines [55]. Disruption of this feedback can aggravate inflammatory conditions, and is found in most underlying autoimmune and inflammatory pathologies, due to a reduced HPA axis response to cytokines or the development of glucocorticoid resistance [55,56], including in FM in which the HPA axis failed to control the increase in pro-inflammatory cytokines [7]. The results presented here support this idea because, after the intervention with the synbiotic, a significant physiological increase in cortisol is observed, together with a decrease in DHEA, particularly in the group without previous diagnosis of CFS, clearly suggesting that the synbiotic generated an activation of the HPA axis (cortisol/DHEA ratio) to compensate for the low-grade inflammation (elevated IL-8) observed in FM patients, also particularly relevant in those without previous diagnosis of CFS. According to some authors [6], the elevated cortisol levels of FM patients is a physiological response to the altered homeostasis caused by their increased inflammatory state. When the stress response is triggered, a negative feedback mechanism is set in motion that protects the body against an "excess" of pro-inflammatory cytokines that can cause tissue damage. This physiological "hormonal" elevation of cortisol following synbiotic consumption may explain the synbiotic induced decrease in IL-8 in the present investigation.

Finally, another question we asked in our research was whether a previous diagnosis of CFS in patients diagnosed with FM could affect the response of the synbiotic. In a previous study conducted in our laboratory CFS codiagnosis does not worsen the subjective perceived psychological and quality of life impairment of FM patients at baseline levels [28]. To the best of our knowledge, this is clearly the first time that the effects of a synbiotic in FM patients (with or without a pre-diagnosis of CFS) have been differentiated. No statistically significant changes were observed in most body composition parameters and physical activity levels during the month-long protocol, as measured objectively by accelerometry. These results suggest that the consumption of the synbiotic did not negatively affect the body composition of the participants during this short period of time, coinciding with another study in which a synbiotic containing probiotics and inulin as a prebiotic was administered [15]. Nevertheless, while the improvement in perceived parameters after taking the synbiotic, such as stress and depression, was more evident in the FM group without a pre-diagnosis of CFS, in perceived fatigue, trait anxiety and fear of COVID-19, the improvement was more evident in the group of FM patients with a pre-diagnosis of CFS. However, as stated before, objective results related to a better regulation of the cytokine-HPA-axis induced by the synbiotic was only found in the group of FM patients without a previous diagnostic of CFS (also with basal elevated levels of IL-8); probably suggesting that women with CFS are, sometimes, over-diagnosed with FM via subjective and perceived evaluations.

A limitation of the present investigation has been the lack of evaluation of the basal level of dysbiosis of FM patients and if the 30 days intervention with the synbiotic is enough to really change the microbiota. Further studies could consider measuring some of the strains characteristic of these two syndromes, before and after treatment with the synbiotic, in order to verify whether a modification of the existing dysbiosis could really be the mechanism responsible for the improved immunoneuroendocrine regulation seen in these patients after consumption of the synbiotic. Although the fact that all the patients who met the inclusion criteria were accepted in the study reinforces the results obtained, future studies with larger numbers of participants, a longer intervention seems to be necessary, particularly found more clearly statistical differences when evaluating FM with or without codiagnosis of CFS separately.

5. Conclusions

In conclusion, the synbiotic seems to have a beneficial effect on the inmunoneuroendocrine imbalance presented by women with FM, provoking a clear response of activation of the HPA axis and subsequently a decrease in the inflammatory profile, an effect that only occurs in patients without a previous diagnosis of CFS. In addition, it produces significant improvements in perceived levels of stress, anxiety and depression, as well as improvements in quality of life during daily activities.

Author Contributions: Conceptualization, E.O. (Eduardo Ortega), M.D.H. and C.D.Q.-C. methodology, E.O. (Eduardo Otero), M.D.H., E.O. (Eduardo Ortega), I.G. and C.D.Q.-C.; formal analysis, E.O. (Eduardo Otero) and C.D.Q.-C.; investigation, M.D.H., E.O. (Eduardo Ortega), C.D.Q.-C. and E.O. (Eduardo Otero); writing—original draft preparation, C.D.Q.-C., M.D.H. and E.O. (Eduardo Otero); writing—review and editing, M.D.H., C.D.Q.-C. and E.O. (Eduardo Ortega); supervision, E.O. (Eduardo Ortega); project administration, E.O. (Eduardo Ortega); funding acquisition, E.O. (Eduardo Ortega). All authors have read and agreed to the published version of the manuscript.

Funding: This research was partially funded by the Gobierno de Extremadura-Fondo Europeo de Desarrollo Regional, Spain (GR21079).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Bioethics Committee of University of Extremadura, Spain (No. Reg. 73/2021, 09/06/2021).

Informed Consent Statement: Written informed consent has been obtained from the patients to publish this paper.

Data Availability Statement: The raw data supporting the conclusions of the manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

Acknowledgments: We are grateful to the associations of Fibromyalgia and Chronic Fatigue Syndrome belonging to the community of Extremadura: EXISTIMOS (Badajoz, Spain) and Heel España S.A.U. for the supply of the synbiotics. This study is part of a national research stay conceded by the Catholic University of Murcia to Carmen Daniela Quero-Calero carried out at the University of Extremadura.

Conflicts of Interest: The authors declare no conflict of interest.

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Resumen global de resultados y discusión.

La Fibromialgia (FM) es una enfermedad cuya fisiopatología continúa sin esclarecerse, caracterizada fundamentalmente por dolor crónico generalizado junto con otras dolencias (fatiga, hiperalgesia, alodinia). En España, la prevalencia estimada es del 2.40% y es mucho más predominante en mujeres (4,2% en mujeres y del 0,2% en hombres) (Cabo-Meseguer y cols., 2017). Debido a que actualmente no existen biomarcadores objetivos conocidos que puedan usarse para diagnosticar esta enfermedad, el diagnóstico se basa en criterios clínicos más o menos subjetivos (Marques y cols., 2017; Laroche, 2019). Esta es la principal causa por la cual la FM es comúnmente codiagnosticada con otros síndromes, como es el caso del síndrome de fatiga crónica (SFC). De hecho, un promedio de 55.2% de pacientes con FM están codiagnosticados con SFC (Yunus y cols., 2012). No obstante, entre los potenciales biomarcadores objetivos en el síndrome de FM, nuestro grupo de investigación ha encontrado que, aun sin ser específico de la FM, la mayoría, si no todas, las pacientes con FM presentaban niveles sistémicos elevados de la citocina IL-8 junto con niveles elevados de mediadores de estrés, como el cortisol y noradrenalina (Ortega y cols., 2009) lo que sugirió claramente una desregulación inmunoneuroendocrina en este síndrome (Bote y cols., 2012),

En este contexto, la prescripción de tratamientos específicos o ayudas terapéuticas (sobre todo no farmacológicas) supone un verdadero problema. Una de las ayudas terapéuticas no farmacológicas más reconocidos en el síndrome de FM es el ejercicio físico habitual (EFH). No obstante, entre los potenciales biomarcadores objetivos en el síndrome de FM, nuestro grupo de investigación ha encontrado que, aun sin ser específico de la FM, la mayoría, si no todas, las pacientes con FM presentaban niveles sistémicos elevados de la citocina IL-8 junto con niveles elevados de mediadores de estrés, como el cortisol y noradrenalina (Ortega y cols., 2009) lo que sugirió claramente una desregulación inmunoneuroendocrina en este síndrome (Bote y cols., 2012), Así, entre los mecanismos de efectividad inmunofisiológicos subyacentes a los beneficios del EFH en pacientes con FM, nuestro grupo de investigación ha demostrado también los efectos antinflamatorios y de regulación inmunoneuroendocrina en estas pacientes, que involucran tanto a las citocinas inflamatorias, particularmente la IL-8 a nivel sistémico, y el cortisol junto a otros mediadores de estrés (Ortega y cols., 2009; Bote y cols., 2012; Bote y cols., 2014), consiguiéndose buenos efectos biorreguladores (Ortega, 2016).

Sin embargo, en la FM existe una discordancia entre las evaluaciones subjetivas y las mediciones objetivas de la actividad física (Estévez-López y cols., 2018), por lo que la acelerometría sería el enfoque preferido (Fredson y cols., 2012; Segura-Jimenez y cols., 2019), particularmente en pacientes que pueden estar codiagnosticadas con el SFC. Además, en cuanto a la realización de EFH en pacientes con SFC existe una gran controversia, ya que, en algunos pacientes existe la preocupación de que el ejercicio exacerbe su sintomatología (Yancey y Thomas, 2012).

Por otro lado, una de las estrategias no farmacológicas más comúnmente utilizadas para mejorar la salud y la calidad de vida de pacientes con FM es la suplementación nutricional (Pagliali et al., 2020). Los probióticos, prebióticos y simbióticos podrían ser beneficiosos para la salud, repercutiendo positivamente en la salud gastrointestinal y en la respuesta inmunitaria e inflamatoria, así como en otros parámetros de la salud mental. Actualmente se proponen varios complementos alimenticios para mejorar los síntomas en la FM y el SFC, entre los que destacan los probióticos (Roman y cols., 2017; Roman y cols., 2018; Pagliali y cols., 2020; Cardona y cols., 2021) y los simbióticos (Pareja y cols., 2015), siendo la investigación sobre estos últimos muy escasa en la literatura científica. Además, las estrategias nutricionales pueden adquirir especial importancia en pacientes con baja adherencia a programas de ejercicio debido a sus dificultades para realizar actividades físicas en programas largos. Sin embargo, hay poca investigación sobre el uso de probióticos en personas diagnosticadas de FM y/o SFC (Sulivan y cols. 2009; Roman y cols., 2018; Cardona y cols., 2021) y son prácticamente inexistentes tras el consumo de simbióticos como ya hemos comentado.

La escasa literatura publicada en relación a cómo afecta la comorbidad de SFC en la vida cotidiana de pacientes con FM nos llevó a diseñar este estudio; con el principal objetivo de evaluar diferencias en la respuesta psicoinmunoneuroendocrina, la calidad de vida asociada al dolor, al estrés, y la fatiga asociada a los niveles de actividad física y sedentarismo, en pacientes diagnosticados con FM, con o sin un codiagnóstico previo de SFC. Se utilizó un grupo de referencia de mujeres del mismo rango de edad no diagnosticadas de FM, SFC, ni ninguna otra patología reumática o de carácter inflamatorio. Este estudio diferencial está justificado en el contexto de los problemas en cuanto a la capacidad disminuida de realización de actividad diaria que presentan estas pacientes, como consecuencia del dolor y otras afecciones nerviosas. Entendemos que puede contribuir a validar y objetivar los aspectos más subjetivos y de calidad de vida de las pacientes. Para alcanzar los objetivos propuestos, se describe brevemente a continuación la metodología utilizada en cada uno de los estudios, cuyos resultados y discusión se resumen posteriormente en este apartado; pudiéndose consultar más detalladamente en las correspondientes publicaciones que constituyen la presente tesis por compendio de publicaciones.

El diseño y número de pacientes utilizados, tanto para la valoración subjetiva o percibida de los parámetros evaluados de forma percibida como para la determinación objetiva de biomarcadores inmunofisiológicos y de actividad física/sedentarismo se resume a continuación (Figura 1):

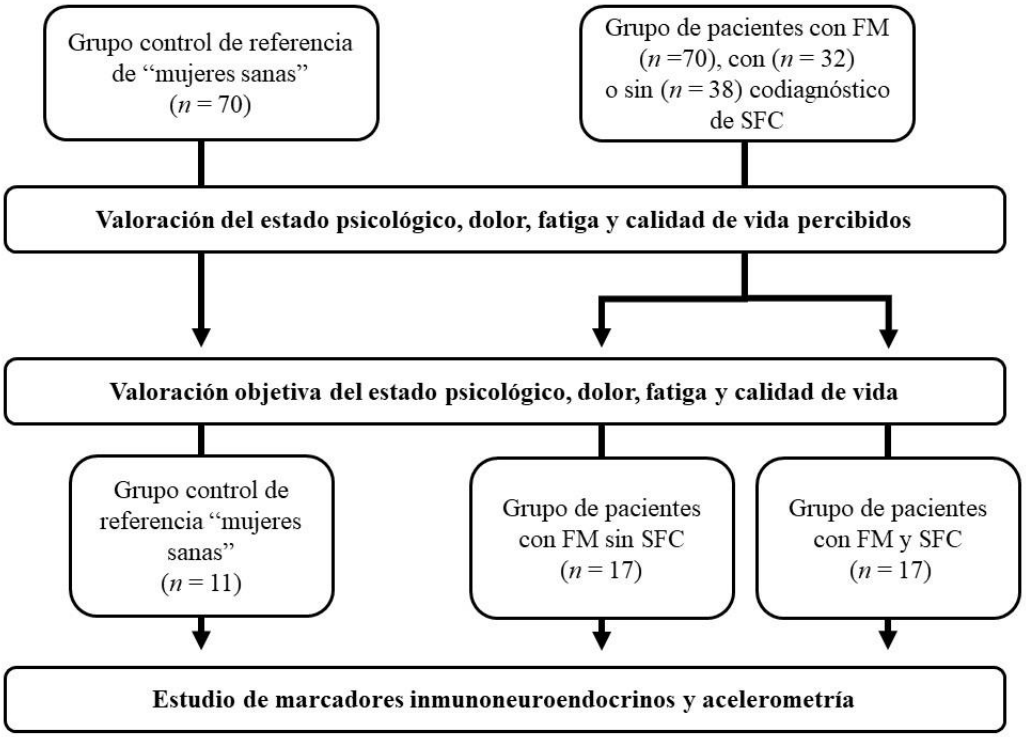

***Figura 1**. Representación esquemática del diseño experimental.*

Cabe destacar que, en la totalidad de publicaciones que componen esta tesis doctoral, se cumplieron los siguientes criterios de inclusión para la conformación de los dos grupos experimentales utilizados; pacientes con FM con o sin un codiagnóstico previo de SFC:

- Mujeres diagnosticadas con FM (de acuerdo con los criterios de la American College of Rheumatology (Wolfe y cols., 2016)) con o sin un codiagnóstico previo de SFC (de acuerdo con los criterios de Fukuda y colaboradores (1994)) por reumatólogos o profesionales de medicina interna.
- Pertenecer a las asociaciones de FM de Extremadura.
- Edad comprendida entre los 40-65 años
- No estar diagnosticado de depresión mayor.
- No padecer sensibilidad química múltiple
- No tener una prescripción de tratamiento con corticoides ni terapias anti-citocinas.

De igual manera, el grupo de referencia de mujeres "sanas" del mismo rango de edad utilizado en los tres estudios también compartía los siguientes criterios de inclusión:

- Mujeres con edad comprendida entre los 40-65 años.
- No estar diagnosticadas de FM ni SFC, ni padecer alguna patología relevante (particularmente de carácter inflamatorio) durante el momento de las valoraciones
- No tener una prescripción de tratamientos farmacológicos que pudieran afectar de forma significativa a las valoraciones de los parámetros de estrés e inflamación determinados.

En primer lugar, para evaluar cómo afecta el codiagnóstico de SFC y la realización de EFH (definido en el estudio como cualquier tipo de actividad física de carácter terapéutico, programada y supervisada, que se realiza un mínimo de dos horas a la semana) a la salud psicológica y a la calidad de vida percibida en pacientes con FM, se procedió a la valoración de la calidad de vida percibida a través de cuestionarios validados científicamente relativos al estrés, ansiedad, depresión, dolor y fatiga; así como al impacto de la enfermedad sobre la ansiedad/miedo frente a la COVID-19. Todo ello se llevó a cabo en dos grupos experimentales de pacientes con FM, con o sin SFC codiagnosticada, utilizando como grupo de referencia un grupo de "mujeres sanas" del mismo rango de edad no diagnosticadas de ninguno de estos síndromes.

La cumplimentación de los cuestionarios se realizó de forma supervisada y con las correspondientes indicaciones de forma y tiempo de éstos. Cabe mencionar, como fortaleza del estudio, que fueron seleccionadas todas las pacientes pertenecientes a las asociaciones de FM de Extremadura que cumplieron con los criterios de inclusión. Extremadura es una Comunidad Autónoma con una población muy homogénea en términos de estilo de vida y, a su vez, se trata de una de las Comunidades Autónomas de referencia en España en investigación biosanitaria en salud pública (Benavent y cols., 2011; Félix-Redondo y cols., 2011), especialmente también en pacientes con FM (Ortega y cols., 2009).

Como era de esperar, las pacientes con FM presentaron una peor salud mental e impacto de la enfermedad frente al grupo de referencia de mujeres controles, sin influencia del codiagnóstico de SFC. Por otro lado, paradójicamente, más de la mitad de las pacientes con FM y SFC codiagnosticada reportaron realizar EFH, en la misma proporción que el grupo de referencia y por encima de las pacientes sin SFC diagnosticada de forma previa. Esto podría ser debido a que, aunque las pacientes con SFC reportan una sustancial intolerancia al ejercicio (Yancey y Tomas, 2012), el tratamiento farmacológico podría ser menos efectivo y más difícil de prescribir que en pacientes con FM sin SFC diagnosticada y, como consecuencia, utilicen con más frecuencia estrategias no farmacológicas como única alternativa para la mejora de su calidad de vida. En este contexto, pudimos comprobar que las pacientes con FM que reportaron EFH presentaron, en general, niveles más saludables de calidad de vida percibida, sin una gran influencia del codiagnóstico de SFC. Únicamente encontramos un efecto diferencial del codiagnóstico de SFC en los parámetros relativos a la depresión, los cuales mejoran únicamente

en pacientes con FM sin SFC diagnosticada que reportan realizar EFH. Estos hallazgos concuerdan con los recientemente publicados por Larun y colaboradores (2017), quienes encontraron que el ejercicio físico no mejoraba la depresión percibida en pacientes con SFC.

Debido a la situación de pandemia por la que hemos pasado, también decidimos evaluar la ansiedad y el miedo frente a la COVID-19, ya que una de las principales características de las mujeres con FM es la menor capacidad para manejar situaciones estresantes, y ello podría intensificar los niveles de depresión y ansiedad (Mohabbat y cols., 2020). En nuestro caso, pudimos comprobar como las pacientes que reportaron realizar EFH manifestaron menos niveles de ansiedad y miedo frente a la COVID-19, con respecto al grupo de referencia. Estos resultados están respaldados por otros estudios que determinaron que una alta proporción de mujeres diagnosticadas con FM agravaron sus síntomas por causa de la ausencia de ejercicio durante la pandemia (Martins y cols., 2021).

Finalmente, aunque el dolor es el síntoma diferenciador y distintivo en las pacientes con FM mientras que en las pacientes con SFC lo es la fatiga, no se encontraron diferencias significativas entre los grupos experimentales en el dolor percibido, pero sí que las pacientes con FM con un codiagnóstico de SFC manifestaron una mayor fatiga percibida. Sin embargo, las pacientes que reportaron realizar EFH no presentaros niveles más saludables ni de dolor ni de fatiga, sin influencia alguna del diagnóstico de SFC.

Como hemos mencionado anteriormente, los problemas existentes en el diagnóstico diferencial subyacen en la ausencia de biomarcadores objetivos que reflejen el estado psicológico de las pacientes. Por tanto, partiendo de la base de que el codiagnóstico de SFC no influye ni en el ya deteriorado estado psicológico ni en la mejora de la calidad de vida debido a la realización de EFH, en esta segunda parte de la investigación nos planteamos profundizar en los trastornos psicoinmunoneuroendocrinos de dichas pacientes y comprobar si un previo diagnóstico de CFS pudiera afectarlos; todo ello determinado tanto de forma percibida, a través de cuestionarios validados científicamente, como de forma objetiva a través de acelerometría y de biomarcadores inmunofisiológicos sistémicos de inflamación y de ansiedad-estrés.

Cabe destacar que, con el fin de conseguir una correcta determinación objetiva tanto de la actividad física (medida mediante acelerometría) como de los parámetros inmunoneuroendocrinos, se extrajeron subgrupos experimentales más reducidos y representativos de cada grupo experimental. Ello nos permitía eliminar las variaciones inter-ensayo: las mediciones de acelerometría se realizaban con los mismos dispositivos en el mismo tiempo y forma, y la determinación de biomarcadores inmunoneuroendocrinos se realizaban en el mismo ensayo experimental. En estos grupos representativos, corroboramos de nuevo que el diagnóstico previo de SFC no afectaba al ya alterado estado psicológico de pacientes con FM.

Uno de los parámetros subjetivos más utilizados para el diagnóstico de FM es la fatiga (Fukuda y cols., 1994). Tanto en FM como en SFC, la fatiga se convierte en el mayor obstáculo, no sólo en la realización de actividad física, sino también en las actividades de la vida diaria (McLoughlin y cols., 2011). Como era de esperar, en la presente investigación observamos de forma objetiva que las pacientes con FM presentan peores niveles de actividad física y mayores niveles de sedentarismo que las personas sanas. Sin embargo, no encontramos una clara influencia del diagnóstico previo de SFC, a pesar de que este último grupo refirió de nuevo mayores niveles de fatiga percibida. Dado que la fatiga objetiva no resultó ser un síntoma diferenciador de estas dos patologías, decidimos centrarnos en los niveles de mediadores neuroinmunoendocrinos (de la inflamación y el estrés) como posibles inductores del dolor objetivo y percibido. La relación entre el dolor y el estrés es bien conocida desde hace tiempo, y nuestro grupo de investigación ya demostró la existencia de una falta de control de la supresión de la respuesta inflamatoria por el cortisol en pacientes con FM que subyacía en una alteración de la interacción entre eje Hipotálamo-Hipófisis-Adrenal (HHA) y el Sistema Nervioso Simpático (SNS) y las citocinas inflamatorias (Bote y cols., 2012). En esta investigación, y en concordancia con dichos resultados, observamos que los altos niveles sistémicos de cortisol encontrados se corresponden con los también altos niveles de estrés percibido en pacientes con FM, con respecto al grupo de referencia. Sin embargo, aunque la influencia del codiagnóstico del SFC en pacientes con FM en el estrés percibido es prácticamente nula, encontramos una menor concentración sistémica de cortisol en dichos pacientes frente al grupo de pacientes con FM sin SFC codiagnosticada, incluso en el mismo rango del grupo de referencia.

Nuestro grupo de investigación había demostrado la existencia de una desregulación en la interacción entre las respuestas inmune/inflamatoria y de estrés en pacientes con FM, particularmente mediada por la IL-8 y el cortisol sistémicos, pero también por otras citocinas inflamatorias liberadas por los monocitos y otros mediadores del estrés, como la noradrenalina (Ortega y cols., 2009; Bote y cols., 2012; García y cols., 2014; Ortega y cols., 2012). Los resultados obtenidos en la presente investigación muestran esta desregulación en la interacción entre la respuesta inflamatoria mediada por IL-8 y la respuesta al estrés mediada por cortisol sólo en pacientes sin diagnóstico previo de SFC. Estos resultados sugerirían que las pacientes con un diagnóstico de SFC podrían estar sobrediagnosticados con FM a través de cuestionarios subjetivos y sin el análisis de biomarcadores objetivos, ya que todas las investigaciones previas de nuestro grupo de investigación reflejaron que las mujeres con FM mostraban niveles elevados de IL-8 en relación con las mujeres sanas (Ortega y cols., 2009). Sin embargo, nuestros resultados no apoyarían la idea de Russel y colaboradores, quienes sugirieron que los niveles de citocinas de IL-1, IL-6 e IL-8 pueden servir como biomarcadores robustos también en la detección del SFC (Russel y cols., 2016).

Además, en relación con los mayores niveles de dolor y depresión declarados por estos pacientes, determinamos las concentraciones sistémicas de noradrenalina y serotonina como biomarcadores objetivos. Encontramos niveles sistémicos más bajos de serotonina en pacientes con FM, que fueron ya descritos en otros estudios (Bote y cols., 2012; Russel y cols., 1992), y concuerdan claramente con los niveles más altos de depresión percibidos por estas pacientes, tanto sin codiagnóstico de SFC como con él. Pero, por otra parte, al igual que con los niveles percibidos de fatiga y actividad física, y con la desregulación neuroinmunoendocrina como mecanismo subyacente al dolor, las pacientes con diagnóstico previo de SFC mostraron concentraciones sistémicas de serotonina más elevadas que las pacientes con FM sin SFC, con valores muy próximos a los del grupo control de mujeres sanas. También ocurre esto en los niveles de oxitocina y noradrenalina, que sólo se encontraron elevados en pacientes con FM sin un codiagnóstico de SFC, resultados que están en consonancia con estudios previos que reflejan concentraciones más elevadas de noradrenalina en pacientes con FM en comparación con mujeres sanas (Ortega y cols., 2009; Bote y cols., 2012). Aunque la noradrenalina en condiciones sanas puede inhibir la liberación de citocinas inflamatorias por las células inmunitarias, se ha descrito que en patologías inflamatorias podría inducir su liberación (Elenkov y cols., 2002), hecho que también se ha indicado en pacientes con FM (Bote y cols., 2012) y que explicaría los resultados de la presente investigación en el contexto de la desregulación neuroinmunoendocrina en esta enfermedad. De nuevo, estos resultados parecen sugerir que el diagnóstico de SFC puede inducir a un sobrediagnóstico de FM a través de síntomas percibidos que, sin embargo, no pueden corroborarse utilizando biomarcadores objetivos. A continuación, se resumen de forma gráfica los resultados de esta parte de la investigación (Figura 2).

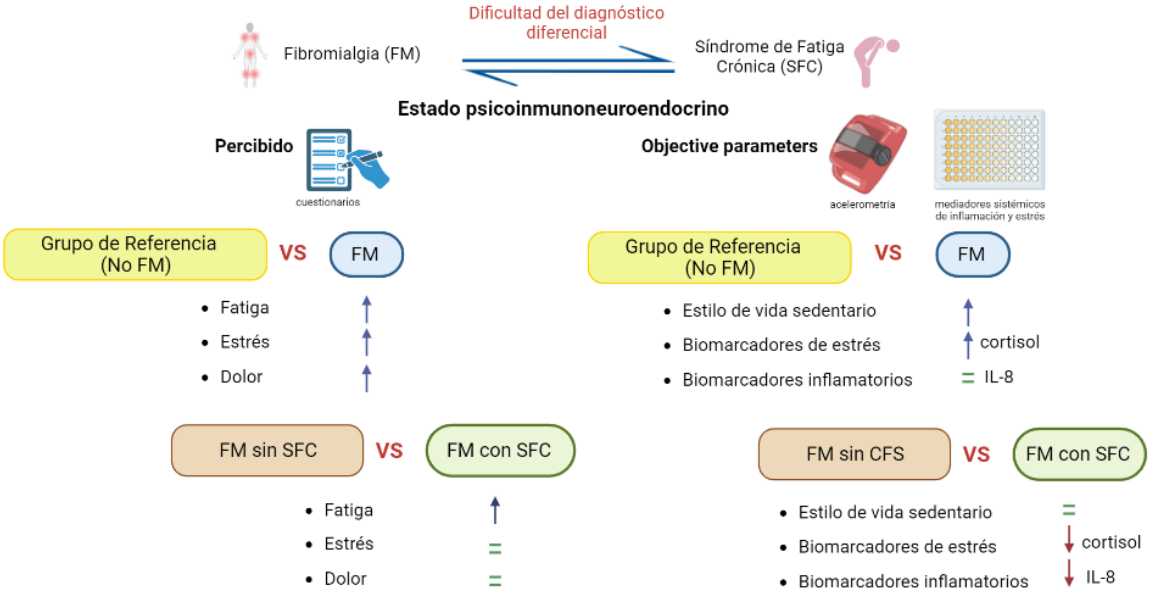

***Figura 2**. Relación entre los trastornos psicoinmunoneuroendocrinos percibidos y objetivos en mujeres con Fibromialgia: influencia del codiagnóstico de Síndrome de Fatiga Crónica. (Adaptado de Otero y cols., 2023)*

Por último, se planteó evaluar los efectos de una intervención con un simbiótico comercializado y de libre consumo (Gasteel Plus®, Heel España S.A.U.) en pacientes con FM y/o SFC codiagnosticada siguiendo los mismos criterios de inclusión que en los estudios anteriores. Todo ello con la finalidad de analizar los posibles efectos de esta intervención en el estado psicoinmunoneuroendocrino de estas pacientes, también mediante el estudio de los mismos biomarcadores objetivos inmunes/inflamatorios y de estrés, así como de los parámetros de salud percibidos y niveles objetivos de actividad física determinados por acelerometría.

Se siguió el mismo protocolo de las valoraciones de la calidad de vida percibida y objetiva actividad física y biomarcadores inflamatorios y de estrés descritos anteriormente. Se realizaron dos evaluaciones (pre- y post- intervención) separadas por 30 días entre ellas, período de tiempo en el cuál deberán consumir el simbiótico. Tras la intervención con el simbiótico, las pacientes con FM (sin y con SFC codiagnosticada) mejoraron su estado de ánimo y sus niveles de estrés y ansiedad percibidos, así como el impacto de la enfermedad en sus actividades diarias determinado subjetivamente mediante cuestionarios científicamente validados. Estos resultados podrían ser coherentes con otras investigaciones (Rao y cols., 2009; Dinan y cols., 2013) que concluyen que, al aumentar el equilibrio microbiano intestinal, los probióticos mejoran la salud del huésped y también pueden beneficiar las funciones cognitivas y psicológicas a través del eje intestino-cerebro (Roman y cols., 2017). En relación a los biomarcadores inflamatorios objetivos, antes de la intervención y al igual que en el estudio anterior, se determinaron altos valores sistémicos de la citocina inflamatoria IL-8; resultados que vuelven a concordar con los publicados anteriormente por nuestro grupo de investigación (Ortega y cols., 2009) y en el estudio correspondiente al segundo objetivo de la presente tesis doctoral anteriormente reseñado (Otero y cols., 2023). Tras la intervención con el simbiótico, los niveles sistémicos de IL-8 disminuyeron en las pacientes con FM (hasta valores compatibles con los de individuos sanos) junto con un aumento de los niveles sistémicos de la citocina antiinflamatoria IL-10, todo ello particularmente evidente en el grupo de pacientes con FM sin codiagnóstico previo de SFC. A su vez, se observó un aumento fisiológico significativo del cortisol tras la intervención con el simbiótico junto con una disminución de la dehidroepiandrosterona (DHEA), particularmente en el grupo sin diagnóstico previo de SFC. Este hecho indica claramente que el simbiótico generó una activación del eje HHA (relación cortisol/DHEA) para compensar la inflamación de bajo grado (IL-8 elevada) observada en las pacientes con FM sin diagnóstico previo de SFC. Por otro lado, no se observaron cambios estadísticamente significativos en la mayoría de los parámetros de composición corporal ni en los niveles de actividad física medidos objetivamente mediante acelerometría. Estos resultados sugieren que el consumo del simbiótico no afectó negativamente a la composición corporal de los participantes durante este corto periodo de tiempo de la intervención, coincidiendo con resultados publicados por otros autores con otro simbióticos en pacientes con FM (Pareja y cols., 2015).

Hasta donde sabemos, ésta es la primera vez que se diferencian los efectos de un simbiótico en pacientes con FM (con o sin un diagnóstico previo de SFC) y, al igual que el estudio anterior, los resultados obtenidos vuelven a sugerir que las pacientes diagnosticadas con SFC pudieran estar en ocasiones sobrediagnosticadas con FM cuando se evalúan únicamente a través de determinaciones percibidas.

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Conclusiones.

A. En relación al primer objetivo, esto es, “evaluar cómo afecta el codiagnóstico de síndrome de fatiga crónica y la realización de ejercicio físico habitual a la salud psicológica y a la calidad de vida percibidas en pacientes con fibromialgia, a través de cuestionarios científicamente validados relativos al dolor, la fatiga, el estrés, la ansiedad, la depresión y la ansiedad frente a la COVID-19”, podemos concluir que:

1. Las personas diagnosticadas con fibromialgia presentan una peor salud psicológica y de calidad de vida percibida que se manifiesta fundamentalmente en peores niveles de estrés, ansiedad, ansiedad frente a la COVID-19, fatiga y dolor con respecto al grupo de referencia de “mujeres sanas” del mismo rango de edad.

No obstante, el diagnóstico previo de síndrome de fatiga crónica (y a excepción de la fatiga, como cabía esperar) no afectó negativamente al ya deteriorado estado psicológico y calidad de vida de las pacientes con fibromialgia.

2. Las pacientes con fibromialgia que reportaron realizar ejercicio físico habitual presentaron mejores estados de estrés y de ansiedad, tanto si presentaban o no un diagnóstico previo de síndrome de fatiga crónica.

Paradójicamente, nuestros resultados no permiten concluir que el codiagnóstico de síndrome de fatiga crónica dificulte la participación en programas regulares y supervisados de actividad física terapéutica, puesto que estas pacientes reportan en mayor medida la realización de estos programas que las pacientes únicamente diagnosticadas con fibromialgia, incluso en el mismo porcentaje de participación al reportado por nuestro grupo de referencia.

B. En relación al segundo objetivo, esto es, “profundizar en los trastornos psicoinmunoneuroendocrinos de las pacientes con fibromialgia y comprobar si un previo diagnóstico de síndrome de fatiga crónica pudiera afectarlos; todo ello determinado tanto de forma percibida, a través de cuestionarios validados científicamente, como de forma objetiva, a través de acelerometría y de biomarcadores inmunofisiológicos sistémicos de inflamación y de ansiedad-estrés”, podemos concluir que:

3. El deterioro de la salud psicológica, la calidad de vida y la fatiga percibidas en las pacientes con fibromialgia se corrobora con las elevadas concentraciones sistémicas de cortisol (sin compensación por elevación de la concentración sistémica de dehidroepiandrosterona) y noradrenalina, así como la menor concentración de serotonina. Igualmente, con los menores niveles de actividad física y mayores niveles de sedentarismo determinados mediante acelerometría; todo ello como marcadores inmunofisiológicos objetivos.

4. A su vez, los mayores niveles de dolor percibido se corresponden también con unas elevadas concentraciones sistémicas de la citocina proinflamatoria IL-8, que no son contrarrestadas por mayores concentraciones de la citocina antiinflamatoria IL-10.

5. Sin embargo, mientras que el diagnóstico previo del síndrome de fatiga crónica no afecta significativamente a las determinaciones objetivas de actividad física y sedentarismo, la mayor respuesta inflamatoria y de estrés valoradas a través de las concentraciones sistémicas de IL-8 y cortisol (desregulación inmunoneuroendocrina) únicamente se observaron en las pacientes sin un diagnóstico previo de síndrome de fatiga crónica. De igual forma, únicamente en este grupo experimental se observan concentraciones sistémicas elevadas de noradrenalina y oxitocina respecto al grupo de referencia y al grupo de pacientes con previo diagnóstico de síndrome de fatiga crónica. Todo lo anterior sugiere un potencial sobrediagnóstico de fibromialgia en las mujeres diagnosticadas de síndrome de fatiga crónica

Únicamente los mayores niveles de adrenalina y de serotonina (hormonas muy relacionadas con la calidad del sueño y el descanso) en las pacientes codiagnosticadas de síndrome de fatiga crónica podrían justificar la mayor fatiga percibida en estos pacientes.

C. En relación al tercer objetivo, esto es, “analizar en pacientes con fibromialgia, con o sin síndrome de fatiga crónica codiagnosticada, los posibles efectos de un simbiótico comercial sobre los parámetros de calidad de vida, inflamación y estrés, tanto de forma percibida como objetiva mencionados en los dos objetivos anteriores”, podemos concluir que:

6. La intervención experimental a través de la ingesta de un simbiótico comercial mejoró los niveles de estrés, ansiedad y depresión percibidos y la calidad de vida asociada a la actividad diaria en las pacientes con fibromialgia, sin afectar a la mayoría de los parámetros de actividad física/sedentarismo evaluados de forma objetiva mediante acelerometría. Estas mejoras fueron particularmente relevantes en las pacientes codiagnosticadas de síndrome de fatiga crónica en el miedo frente a la COVID-19, la ansiedad y la fatiga.

7. Además, y como posible mecanismo inmuofisiológico de la mejora en los parámetros percibidos, el simbiótico provocó una activación del eje Hipotálamo-Hipófisis-Adrenal (incremento de concentración sistémica de cortisol y ratio cortisol/dehidroepiandrosterona) que podría disminuir el elevado estado inflamatorio (disminuyendo la elevada concentración sistémica basal de IL-8 y elevando la concentración de IL-10) en las pacientes con fibromialgia, particularmente en aquéllas sin un diagnóstico previo de síndrome de fatiga crónica. Ello puede reforzar la idea de un posible sobrediagnóstico de fibromialgia en pacientes con síndrome de fatiga crónica.

Conclusión global

Teniendo en cuenta nuestra hipótesis general de partida de que "la comorbidad del síndrome de fatiga crónica en las pacientes con fibromialgia pudiera deteriorar aún más su capacidad para realizar actividad física, sus niveles de estrés y, en definitiva, el dolor y la fatiga que dificultan las actividades cotidianas y, por tanto, la calidad de vida; correlacionándose con un aumento en el desequilibrio de la respuesta psiconeuroinmunoendocrina", podemos concluir de forma global que:

En contra de lo hipotetizado, un codiagnóstico previo de síndrome de fatiga crónica no empeora, de forma general (a excepción de la fatiga), la salud psicológica y calidad de vida percibidas por las pacientes. Esta ausencia de diferencias en la calidad de vida percibida sólo se demuestra de forma objetiva al valorar los niveles de actividad física. Sin embargo, los mayores niveles de estrés y dolor percibidos por estas pacientes sólo pueden sustentarse a través de biomarcadores de inflamación y de estrés (respuesta inmunoneuroendocrina) en aquellas pacientes sin un codiagnóstico de síndrome de fatiga crónica; en las únicas que a su vez también se observó una mejora en la interacción inmunoneuroendocrina objetiva tras la intervención con el simbiótico.

Todo ello en su conjunto, y teniendo en cuenta los errores que toda generalización conlleva, sugiere fuertemente un posible sobrediagnóstico de fibromialgia en pacientes que han sido diagnosticadas con síndrome de fatiga crónica. Por tanto, es crucial la valoración de biomarcadores objetivos, y no únicamente percibidos, para un buen diagnóstico diferencial de estos síndromes.

Anexo I. Comunicaciones a congresos

1. **Otero E**, Hinchado MD, Gálvez I, Perez-Ortega A, Bote ME, Ortega E. Actividad física en pacientes con fibromialgia, con y sin fatiga crónica asociada. Comunicación oral presentada en: I Congreso Internacional en Ciencias de la Actividad Física y el Deporte; 2020 Sept 13-15; Cáceres, España.

2. **Otero E**. Influencia de la comorbilidad del síndrome de fatiga crónica en pacientes con fibromialgia sobre la actividad física, la calidad de vida y la respuesta inmunoneuroendocrina. Comunicación oral presentada en: VI Simposio del Programa de Doctorado de Biomarcadores de Salud y Estados Psicológicos: 2021 Jul 7. Cáceres, España

3. **Otero E**, Hinchado MD, Bote ME, Navarro MC, Gálvez I, Martín-Cordero L, Ortega E. Actividad física y calidad de vida en pacientes con fibromialgia, con y sin fatiga crónica asociada. Comunicación oral presentada en: XVIII Congreso Internacional de la Sociedad Española de Medicina del Deporte (SEMEDE); 2021 Nov 25-27; Murcia, España. Abstract publicado en: *Archivos de Medicina del Deporte* (2021) nº206 38(6) 403–429.

4. Hinchado MD, **Otero E**, Marín J, Bote ME, Martín-Cordero L, Gálvez I, Navarro MC, Ortega E. Influencia del síndrome de fatiga crónica en la actividad física y estado psicológico de pacientes con fibromialgia. Comunicación oral presentada en: XVIII Congreso Internacional de la Sociedad Española de Medicina del Deporte (SEMEDE); 2021 Nov 25-27; Murcia, España. Abstract publicado en: *Archivos de Medicina del Deporte* (2021) nº206 38(6) 403–429.

5. **Otero E**, Martín-Cordero L, Navarro MC, Gálvez I, Ortega E, Hinchado MD. Influence of regular physical exercise on the psychological state of fibromyalgia patients, with and without a co-diagnosis of chronic fatigue syndrome. Póster presentado en: The 4th International Virtual Congress on Controversies in Fibromyalgia; 2022 May 25-26. Online, organized by Bioevents ®. Abstract publicado en: Ablin, J. N., Sarzi-Puttini, P., & May 25-26, 2022 (2022). The 4th International Virtual Congress on Controversies in Fibromyalgia. *Clinical and experimental rheumatology*, 40(6), 1225–1246. https://doi.org/10.55563/clinexprheumatol/ajiygc

6. **Otero E**, Martín-Cordero L, Navarro MC, Gálvez I, Ortega E, Hinchado MD. Influence of co-diagnosis of chronic fatigue syndrome on the psychological state and life quality of fibromyalgia patients. Póster presentado en: The 4th International Virtual Congress on Controversies in Fibromyalgia; 2022 May 25-26. Online, organized by Bioevents ®. Abstract publicado en: Ablin, J. N., Sarzi-Puttini, P., & May 25-26, 2022 (2022). The 4th International Virtual Congress on Controversies in Fibromyalgia. *Clinical and experimental rheumatology*, 40(6), 1225–1246. https://doi.org/10.55563/clinexprheumatol/ajiygc

7. **Otero E**. Objetivación de los niveles de calidad de vida percibida a partir de biomarcadores inmunoneuroendocrinos en pacientes con Fibromialgia, con y sin un codiagnóstico de Síndrome de Fatiga Crónica. Comunicación oral presentada en: VII Simposio del Programa de Doctorado de Biomarcadores de Salud y Estados Psicológicos: 2022 Jul 4. Badajoz, España

8. Hinchado MD, **Otero E**, Navarro MC, Martín-Cordero L, Gálvez I, Ortega E. Influence of codiagnosis of chronic fatigue syndrome on the perceived and physiological stress of fibromyalgia patients. Presentado en: XL Congreso de la Sociedad Española de Ciencias Fisiológicas (SECF); 2022 Sept 19-22; Badajoz, España. Abstract publicado en: Abstracts of the XL Congress of the Spanish Society of Physiological Sciences (SECF). (2022). *Journal of physiology and biochemistry*, 78(Suppl 1), 1–99. Advance online publication. https://doi.org/10.1007/s13105-022-00923-3

9. **Otero E**, Hinchado MD, Navarro MC, Gálvez I, Martín-Cordero L, Ortega E. Niveles objetivos de actividad física habitual en pacientes con fibromialgia: influencia del codiagnóstico del síndrome de fatiga crónica. Comunicación oral presentada en: X Jornadas Internacionales de la Sociedad Española de Medicina del Deporte (SEMEDE); 2022 Nov 25-26. Abstract publicado en: *Archivos de Medicina del Deporte* (2023) nº213 40(2) 49-62.