Tesis doctoral

UNIVERSIDAD DE GRANADA
Instituto de Neurociencias "Federico Olóriz"

The effects of gluten-free diet versus hypocaloric diet among patients with fibromyalgia experiencing gluten sensitivity symptoms: Pilot, open-label, randomized clinical trial

Mahmoud Slim

Granada, 2015

UNIVERSIDAD DE GRANADA
FACULTAD DE MEDICINA
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Ensayo clínico abierto y aleatorizado comparando dieta sin gluten con dieta hipocalórica en pacientes con fibromialgia

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Que el trabajo de investigación titulado: "The effects of gluten-free diet versus hypocaloric diet among patients with fibromyalgia experiencing gluten sensitivity symptoms: Pilot, open-label, randomized clinical trial " ha sido realizado por D. Mahmoud Slim para optar al grado de Doctor por la Universidad de Granada, en el instituto de Neurociencias de la Universidad de Granada, bajo mi dirección.

Y para que conste donde proceda se firma este certificado en Granada a 19 de Mayo de 2015

Fdo. Elena Pita Calandre

Resumen

Introducción: El síndrome de Fibromialgia, un trastorno de dolor crónico musculoesquelético, presenta una prevalencia de 2-5% en la población general. Se trata de una patología compleja con un amplio espectro de síntomas, entre los que destacan el cansancio crónico, el sueño no reparador y los problemas cognitivos. También son frecuentes síntomas gastrointestinales, parcialmente atribuibles a la comorbilidad entre la fibromialgia y el síndrome de intestino irritable que se estima entre 32 y 81%. Sin embargo, una proporción alta de pacientes con fibromialgia presentan con síntomas gastrointestinales inespecíficos que no son suficientes para cumplir los criterios diagnósticos del síndrome del intestino irritable ni para ser adscritos a otro trastorno digestivo específico. Destacan entre ellos la alternancia diarrea-estreñimiento, la distensión abdominal, la dispepsia, las náuseas, el estreñimiento o la diarrea, desconociéndose si esta sintomatología inespecífica es debida a una patología comórbida o si forman parte del espectro sintomatológico del propio síndrome fibromiálgico. Por otro lado, estos síntomas de fibromialgia (tanto gastrointestinales como extraintestinales) guardan similitud con el espectro de síntomas presentes en la enfermedad celíaca y en la sensibilidad al gluten no celiaca. Esta superposición sintomatológico sugiere un posible papel de la sensibilidad al gluten por lo menos en un subgrupo de pacientes con fibromialgia que presentan manifestaciones gastrointestinales. Por ello, el objetivo del presente estudio consiste en comparar la eficacia y tolerabilidad de una dieta exenta de gluten (DSG) respecto a una dieta hipocalórica (DHC) en pacientes con fibromialgia que muestran síntomas relacionados a la sensibilidad del gluten.

Metodología: Se realizó un ensayo clínico de 24 semanas, aleatorizado y abierto que incluyó pacientes con fibromialgia, diagnosticados según los criterios de American College of Rheumtalogy 2010, y mostraron síntomas de sensibilidad al gluten. Se excluyeron los pacientes con anticuerpos tranglutaminasa positiva, o que sufriera alguna enfermedad que pudiera interferir el seguimiento correcto de las terapias dietéticas anticipadas. Los pacientes fueron asignados aleatoriamente a una dieta sin gluten o una dieta hipocalórica. El objetivo primario fue evaluar el cambio en el número

de síntomas relacionados a la sensibilidad al gluten. Los objetivos secundarios incluyeron los cambios en las medidas antropométricas, el Cuestionario de Impacto de Fibromialgia revisada (FIQR), el Índice de Calidad del Sueño de Pittsburgh (PSQI), el Inventario Breve de Dolor (BPI), el Inventario de Depresión de Beck (BDI-II) y el Cuestionario de salud SF-12. Asimismo, se evaluaron la Impresión Clínica Global de Severidad y de Mejoría evaluada por el paciente (PGI-S y PGI-I) y las reacciones adversas. Para el análisis estadístico, se empleó la muestra denominada "por intención de tratar" y se aplicó el método LOCF (Last Observation Carried Forward)**,** de forma que la última evaluación disponible del paciente se repite en las evaluaciones posteriores. La evolución de los pacientes, se analizó mediante análisis de varianza (ANOVA) de dos vías. Las diferencias en el cambio entre ambos grupos se realizaron con la t de Student. Se utilizó el test de Chi cuadrado para valorar datos cualitativos.

Resultados: Un total de 81 sujetos fueron seleccionados como potencialmente elegibles, de los cuales 79 cumplieron con los criterios de inclusión. Setenta y cinco de los 79 sujetos fueron aleatorizados para recibir DSG (n = 35) o DHC (n = 40). La mayoría de los participantes del estudio eran mujeres (97%) con una edad media de 51,25 ± 8,13. Se observaron características demográficas basales similares en ambos grupos. Hubo un efecto significativo de tiempo sobre el cambio en los síntomas relacionados a la sensibilidad al gluten (-2,46±0,4 en DSG; -2,1±0,38 en DHC; $p<0,0001$), pero la diferencia media del cambio entre los dos grupos no fue estadísticamente diferente. Se observó un efecto significativo de tiempo y tratamiento sobre el cambio en el índice de masa corporal (-0,75±0,2 en DSG; -1,2±0,2 en DCH), pero los tamaños del efecto en ambos grupos fueron pequeños. A pesar de que se logró solo un efecto significativo de tiempo en el cambio del FIQR (-9,2±2,1 en DSG; -8,7±2,9 en DHC; $p<0,0001$), los cambios intragrupos fueron clínicamente relevantes con tamaños del efecto. También se observó un efecto significativo del tiempo en los cambios del PSQI, el BPI y el BDI-II. Se observó un efecto clínicamente relevante en el cambio del componente físico del SF-12 en los pacientes asignados a la DHC. El 43% de los pacientes asignados a la DSG y el 50% de los asignados a la DHC refirieron mejoría al acabar el estudio. Ambas intervenciones dietéticas fueron bien toleradas.

Conclusiones: Ambas dietas disminuyeron el número de síntomas relacionadas a la sensibilidad al gluten. Pero, a pesar de su especificidad, la DSG no fue más eficaz que la DHC. El papel de las terapias dietéticas en el tratamiento de la fibromialgia es importante y requiere más investigación. Ya que la ausencia de ensayos clínicos controlados adecuadamente diseñados para investigar el papel de las intervenciones dietéticas en la fibromialgia impide recomendarlas de forma concluyente.

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1. Introduction

1.1. Fibromyalgia: A road hedged up with thorns

Fibromyalgia is a chronic widespread pain syndrome characterized by a complex nature and wide array of signs and symptoms. Both somatic and psychological manifestations characterize this syndrome. The cardinal symptom that defines fibromyalgia is chronic generalized musculoskeletal pain without an underlying identifiable cause. However, although pain is a prominent feature of the fibromyalgia syndrome, other disabling symptoms coexist and contribute to a more complex clinical presentation. Sleep disturbances and chronic fatigue are present in almost all patients with fibromyalgia; many patients also report cognitive difficulties, stiffness, balance problems, psychological abnormalities (depression and/or anxiety) and hypersensitivity to environmental stimuli (Arnold et al. 2011). The evolution of fibromyalgia and its recognition as a discrete syndrome has been a debatable issue, despite its recognition as a discrete medical illness by the American Medical Association in 1987 (Goldenberg 1987), the American College of Rheumatology (ACR) in 1990 (Wolfe et al. 1990), the “Copenhagen declaration” in 1992 (Csillag 1992), the World Health Organization (WHO) in 1992 (World Health Organization 1992) and the international association for the study of pain (IASP) in 1994 (IASP 1994) .

Skepticism about its distinct nature arises from the subjective nature of chronic pain, the variable tender point examination, the absence of a gold standard laboratory test or biomarker, and the unclear pathophysiologic mechanisms (Rau and Russell 2000). Some healthcare professionals had considered that the recognition of fibromyalgia as a distinct entity was only an attempt to create a homogeneous clinical entity out of the musculoskeletal pain phenomena for investigational purposes (Cohen and Quintner 1993). However, the practical violation of these criteria as claimed by Cohen has turned fibromyalgia into a tautological concept that can be shown to be true as it includes all possibilities; the elusive tender points in the absence of clinicophysiological explanation implicate a circular argument upon which the appreciation of fibromyalgia as an independent entity was based (Cohen and Quintner 1993, Cohen 1999). Wessely and Hotopf assert that various medical specialties have defined syndromes to categorize

patients with unexplained medical symptoms, such as the irritable bowel syndrome defined by gastroenterologists, the non-cardiac chest pain (Syndrome X) defined by cardiologists and similarly did rheumatologists in devising fibromyalgia (Wessely and Hotopf 1999).

Despite of the subjectivity governing fibromyalgia, healthcare professionals in favor of the distinctive nature of this syndrome claim that sufficient objective findings have been provided concerning the characterization of fibromyalgia as a discrete entity. Well documented abnormalities of several physiological mechanisms have been described such as abnormalities in the neuroendocrine mechanisms, autonomic nervous system, nociceptive processing and pronociceptive mediators' abnormalities (Fitzcharles 1999, Russell 1999, Perrot 2012). Fitzcharles and Yunus consider that various neurophysiological studies have contributed to the acceptance of fibromyalgia as a discrete syndrome, and the dysregulation of pain processing in fibromyalgia has been proven to be present at different levels in the nervous system (Fitzcharles and Yunus 2012). Other findings that support the recognition of fibromyalgia as a definite syndrome are the recent identification of certain genetic mutations that predispose individuals to fibromyalgia, in addition to the neurobiological studies showing abnormalities within central brain structures (Harris and Clauw 2006).

The disconnection between any complaint and the physical examination findings usually generates skepticism among clinicians; this was also evident in the construct of phantom limb syndrome, which is now accepted as a real discrete syndrome causing pain in the absence of anatomical tissue in the periphery (Fitzcharles and Yunus 2012). In response to the skepticism originating from the absence of any objective biomarkers corresponding to fibromyalgia, Fitzcharles and Yunus point toward depression which is an undeniable serious condition but yet lacks any objective biomarkers (Fitzcharles and Yunus 2012).

Despite the controversy surrounding the characterization and recognition of fibromyalgia, the entire medical community currently acknowledges the existence of this syndrome. After three decades following the recognition of fibromyalgia as a discrete

illness with specific diagnostic criteria, considerable progress in medical investigation has been achieved, contributing to a better understanding of this syndrome and to the gradual unveiling of the obscured mechanisms underlying this disease, although a complete understanding of fibromyalgia is not yet fulfilled. Consequently, less suspicion is currently encountered in the healthcare society regarding the appreciation of fibromyalgia. This also has led to channeling the discussions from arguing the existence of this syndrome toward focusing on the full comprehension of the exact underlying mechanisms and, accordingly, designing the optimal therapeutic treatment plan.

1.2. Historical Evolution

The current description of fibromyalgia is the outcome of a long process of historical evolution extending over a period of several centuries. Many medical terms throughout history were suggested to best describe this complex spectrum of manifestations.

The first description of the clinical manifestations corresponding to muscular pain and rheumatic fever dates back to 1592 by the French physician Guillaume de Baillou (Ruhman 1940). The term “muscular rheumatism” was applied to painful but non-deforming musculoskeletal disorders to be distinguished from articular rheumatism. Subsequent efforts to understand and describe muscular rheumatism emerged in the 1800s. The British physician William Balfour was among the pioneers to suggest the presence of an underlying inflammation in the connective tissues contributing to the appearance of nodules and pain and he was the first to describe tender points associated to fibromyalgia (Inanici and Yunus 2004).

Emphasizing the inflammatory nature of the disease, Sir William Gowers coined the term “fibrositis” in 1904 to describe the inflammation of fibrous tissue; a term which was constantly used to describe the disease for the following 72 years. In his article, Gowers claimed that “the conception of muscular rheumatism as a rheumatic inflammation is, I know, old enough, and the term "fibrositis” is so convenient that I cannot doubt that it has been used.” (Gowers 1904). Conforming to the assertion of Gowers, Stockman provided the pathologic grounds for fibrositis as he described the presence of patchy inflammatory changes in the fibrous tissue from seven biopsy studies of “fibrositic

nodules". However, subsequent pathologic studies failed to reproduce Stockman's findings and to encounter any evidence of low grade inflammatory process and subsequently the term "fibrositis" was later considered a misnomer (Bennett 1981).

The second part of the 20th century witnessed a breakthrough progress in defining the basic concepts of the modern fibromyalgia. An important contribution that led to a better understanding of fibromyalgia was made by Traut in 1968 who described generalized fibrositis to be characterized by generalized stiffness and aching, anxiety, fatigue, headaches, sleep disturbances and colitis. Moreover, the specific tender points' sites were illustrated by Traut who also assumed axial pain to be an important criterion for the classification of fibrositis (Inanici and Yunus 2004). This was followed by the substantial contributions of Smythe who described fibromyalgia as a generalized pain syndrome accompanied by fatigue, morning stiffness, sleep disturbances, multiple tender points and emotional distress and thus introducing the first diagnostic criteria specific to fibromyalgia in 1972 (Smythe 1972). In 1975, the Canadian psychiatrist Moldofsky reported, using sleep electroencephalogram (EEG), an abnormality in the stage 4 of sleep (absent) in fibrositis (Moldofsky et al. 1975). One year later, the same author reported the ability to induce the symptoms of fibromyalgia in healthy subjects through sleep deprivation emphasizing the "non-restorative sleep" concept in fibromyalgia (Moldofsky and Scarisbrick 1976). Modifications to the initial diagnostic criteria suggested by Smythe in 1972 were published in 1977 by Smythe and Moldofsky (Smythe and Moldofsky 1977). These criteria required the presence of widespread pain for more than 3 months, fatigue, stiffness, sleep disturbance and tenderness in at least 11 out of 14 pre-specified tender points (Smythe and Moldofsky 1977).

A fundamental contribution to the understanding of fibromyalgia appeared in 1981 through the controlled clinical trial conducted by Yunus et al. (1981). The outcomes of this study led to the suggestion of a new set of diagnostic criteria for fibromyalgia with 96% sensitivity and 100% specificity (Yunus et al. 1981). These criteria were commonly used by investigators until the appearance of the ACR diagnostic criteria in 1990 (Bengtsson 2002).

It was not until 1990 that the first official classification criteria for fibromyalgia were developed by the American College of Rheumatology (Wolfe et al. 1990). Thereafter, fibromyalgia was recognized as a distinct syndrome and the ACR criteria were applied to subsequent studies and clinical trials that markedly improved the knowledge about the disease.

1.3. Diagnostic Criteria

The diagnosis of fibromyalgia is established based on the clinical evaluation of the patients. As stated above, initial diagnostic criteria were proposed by Smythe in 1972 and 1977 (Smythe 1972, Smythe and Moldofsky 1977) followed by the set of diagnostic criteria suggested by Yunus et al. in 1981 (1981). However, it was not until 1990 that the first official diagnostic criteria for fibromyalgia were suggested by the ACR (Wolfe et al. 1990). With a sensitivity of 88.4% and specificity of 81.1%, the ACR 1990 criteria for the classification of fibromyalgia required the presence of:

a. History of widespread pain for a minimum duration of 3 months. According to the ACR criteria, “pain is considered widespread when all of the following are present: pain in the left side of the body, pain in the right sideof the body, pain above the waist and pain below the waist. In addition, axial skeletal pain (cervical spine or anterior chest orthoracic spine or low back) must be present. In this definition, shoulder and buttock pain is considered as pain for each involved side. "Low back" pain is considered lower segment pain”.
b. Pain in 11 out of 18 tender point sites on digital palpation which should be performed with an approximate force of 4 kg. For considering any tender point as “positive”, the subject must state that the palpation was painful. The 18 tender points are located in the following sites:
    i. Occiput: bilateral, at the suboccipital muscle insertions.
    ii. Low cervical: bilateral, at the anterior aspects of the intertransverse spaces at C5-C7.
    iii. Trapezius: bilateral, at the midpoint of the upper border.

iv. Supraspinatus: bilateral, at origins, above the scapula spine near the medial border.
v. Second rib: bilateral, at the second costochondral junctions, just lateral to the junctions on upper surfaces.
vi. Lateral epicondyle: bilateral, 2 cm distal to the epicondyles.
vii. Gluteal: bilateral, in upper outer quadrants of buttocks in anterior fold of muscle.
viii. Greater trochanter: bilateral, posterior to the trochanteric prominence.
ix. Knee: bilateral, at the medial fat pad proximal to the joint line.

These tender points are illustrated in the following scheme (Figure 1):

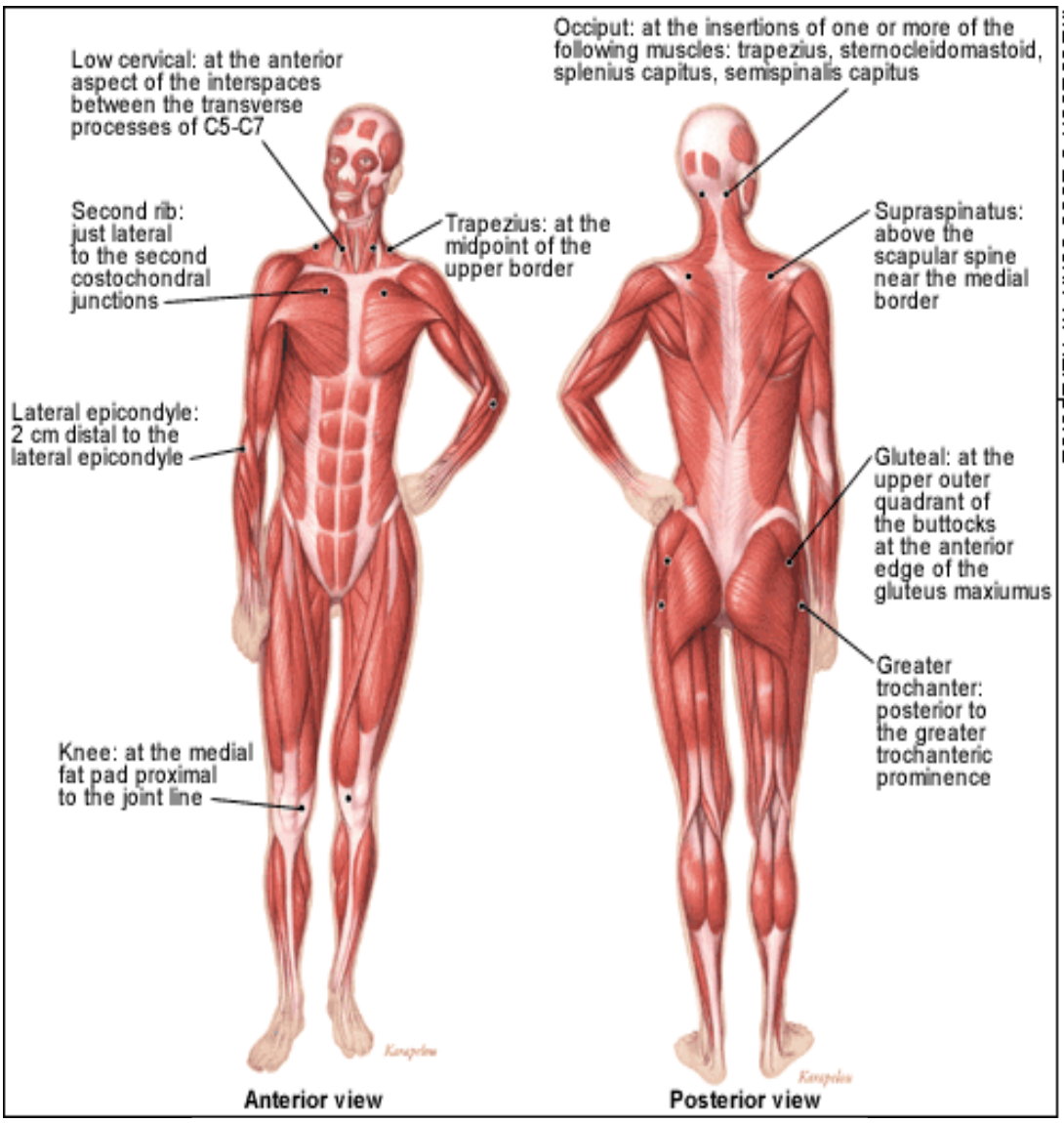


Figure 1 Tender points distribution in fibromyalgia

Adapted from Millea PJ et al. *Am Fam Phys 2000.*

As it can be noticed, these criteria solely relied on pain to establish a diagnosis of fibromyalgia. As reported by the authors, tender points were the most powerful discriminator for the diagnosis of fibromyalgia. Individual symptoms such as sleep disturbance, fatigue and morning stiffness had good discriminating power; however, the simultaneous occurrence of these manifestations was only seen in 56% of patients (Wolfe et al. 1990). Therefore, despite their relevance, their simultaneous presence did not seem to improve diagnostic accuracy in fibromyalgia and thus, they were not endorsed among the diagnostic criteria.

These criteria have been adopted in clinical practice over a period of 20 years before the appearance of the 2010 diagnostic criteria. However, during this period of time several objections to them appeared. For instance, examining the designated tender points required previous training and experience. Consequently, many physicians refused to carry out the tender point examination due to their lack of sufficient clinical experience necessary for undertaking this task and therefore diagnosis was mainly made based on the presence of symptoms (Wolfe et al. 2010).

Moreover, the importance of symptoms that had not been considered among the 1990 diagnostic criteria started to be better recognized as key fibromyalgia features such as fatigue, cognitive symptoms and the extent of somatic symptoms. Another important consideration was in viewing fibromyalgia as a spectrum disorder which was not well served by dichotomous criteria of the ACR 1990. In addition, many patients, who experienced improvement in fibromyalgia symptoms and tender points count, could fail to satisfy the ACR 1990 classification despite the fact that fibromyalgia syndrome is still there and no remission is experienced. These two facts suggested the need of a severity scale capable of classifying patients with fibromyalgia according to the extent of severity of their symptoms (Wolfe et al. 2010).

Accordingly, with an aim to formulate non-tender point diagnostic criteria for fibromyalgia and to integrate severity scale-based symptoms of the characteristic features of fibromyalgia, the ACR published the second set of preliminary diagnostic criteria for fibromyalgia in 2010 (Wolfe et al. 2010). These criteria were not intended to replace the old ones but rather they represent an alternative method of diagnosis (Wolfe et al. 2010). They substituted the tender point evaluation by a Widespread Pain Index (WPI) and added a Symptom Severity (SS) Scale that quantifies fatigue, waking unrefreshed, and cognitive symptoms. The new criteria also take into consideration the presence of other somatic symptoms.

According to the ACR 2010 diagnostic criteria, a patient is diagnosed with fibromyalgia if the following 3 conditions are met:

a. WPI ≥7 and SS scale ≥5 or WPI 3-6 and SS scale ≥9.

b. Symptoms have been present at a similar level for at least 3 months.
c. The patient doesn't have a disorder that otherwise explain the pain.

The WPI is used to assess the number of areas in which the patient has had pain over the last week. Nineteen different sites are evaluated as such score will be between 0 and 19: left and right shoulder girdle, left and right upper arm, left and right lower arm, left and right Hip (buttock, trochanter), left and right upper leg, left and right lower leg, left and right jaw, chest, abdomen, upper and lower back, neck.

SS scale score is the sum of the severity of the 3 symptoms (fatigue, waking unrefreshed and cognitive symptoms) and the extent (severity) of somatic symptoms. The final score is between 0 and 12.

a. The level of severity of the 3 symptoms, over the past week, is evaluated according to the following:
    i. 0= No problem
    ii. 1= slight or mild problems, generally mild or intermittent
    iii. 2= moderate, considerable problems, often present and/or at a moderate level
    iv. 3= severe: pervasive, continuous, life-disturbing problems
b. The extent of somatic symptoms is evaluated according to the following:
    i. 0= no symptoms
    ii. 1= few symptoms
    iii. 2= a moderate number of symptoms
    iv. 3= a great deal of symptoms

Somatic symptoms that might be considered: muscle pain, irritable bowel syndrome, fatigue/tiredness, thinking or remembering problem, muscle weakness, headache, pain/cramps in the abdomen, numbness/tingling, dizziness, insomnia, depression, constipation, pain in the upper abdomen, nausea, nervousness, chest pain, blurred vision, fever, diarrhea, dry mouth, itching, wheezing, Raynaud's phenomenon, hives/welts, ringing in ears, vomiting, heartburn, oral ulcers, loss of/change in taste, seizures, dry eyes, shortness of breath, loss of appetite, rash, sun sensitivity, hearing

difficulties, easy bruising, hair loss, frequent urination, painful urination, and bladder spasms.

These new set of criteria has been shown to correctly classify 88.1% of cases without the need of physical or tender point examination. The assessment of fibromyalgia symptoms severity in individuals with current or previous fibromyalgia would be now reachable (Wolfe et al. 2010).

Eventually, the 2010 preliminary diagnostic criteria were further modified in 2011 by proposing the new Fibromyalgia Symptom Scale (FS) which combines the WPI and modified-SS scales in an attempt to facilitate self-administration by patients (Wolfe et al. 2011).

1.4. Epidemiology

Studies aiming to identify the prevalence of fibromyalgia in the general population date back to early 1980s. However, these efforts faced a fundamental challenge of unidentified diagnostic criteria or at least the lack of standardized criteria. Despite this fact, a consensus of perceiving fibromyalgia as a prevalent disorder was established. The later publication of the 1990 and 2010 ACR criteria (Wolfe et al. 1990, Wolfe et al. 2010) for the diagnosis of fibromyalgia constituted a substantial progress toward establishing more accurate outcomes at the level of prevalence studies.

Despite of the appearance of the ACR 1990 criteria, the variability in the outcomes of the prevalence studies persisted. While the prevalence of fibromyalgia in a Danish population was estimated to be 0.66% (Prescott et al. 1993), the prevalence in Norway reached 10.5% (Forseth and Gran 1992) and 3.2% in South Africa (Lyddell C 1992). This wide variability in estimating the prevalence of fibromyalgia despite the use of standardized criteria (ACR 1990) could be attributed both to the differing methodological techniques and the distinctive target populations being evaluated.

The elimination of the previous classification of fibromyalgia (primary and secondary) upon the adoption of the ACR 1990 diagnostic criteria reduced the burden that faced earlier epidemiological studies in categorizing patients based on the presence of

concomitant conditions which in turn required the realization of extensive physical, radiographic, and laboratory examinations in order to identify comorbid conditions (Wolfe et al. 1995). In 1995, Wolfe et al. (1995) conducted a community study, which included 3,006 subjects from Wichita (USA), aiming to estimate the prevalence of fibromyalgia and to evaluate its association with several sociodemographic and psychological factors. The estimated prevalence of fibromyalgia was 2.0% for both sexes (95% CI 1.4, 2.7), 3.4% for women (95% CI 2.3, 4.6) and 0.5% for men (95% CI 0.0, 1.0) (Wolfe et al. 1995). The authors of this study demonstrated that the prevalence of fibromyalgia increases with age as the highest prevalence figure was seen among participants who were between 60 and 79 years old (>7% in women). In addition to its strong association with the female gender, fibromyalgia was shown to be associated with several sociodemographic factors, such as lower educational level, being divorced and reduced household income, and psychological factors, such as somatization, anxiety and depression (Wolfe et al. 1995).

Consistent with the findings reported by Wolfe et al. (1995), the London fibromyalgia epidemiology study conducted in Ontario (Canada) reported an overall prevalence of 3.3% among 3,395 non-institutionalized adults (White et al. 1999). The mean age for patients with fibromyalgia was reported to be 49.2 years in females and 39.3 in among males. A considerable gender difference was seen for the prevalence of fibromyalgia (4.9% in females versus 1.6% in males). In this study, the peak prevalence estimate was seen in women between 55 and 64 years (8%) (White et al. 1999).

In Spain, the lack of reliable estimates concerning the prevalence of specific rheumatic diseases (low back pain, rheumatoid arthritis, osteoarthritis and fibromyalgia) urged the Spanish Society of Rheumatology to realize a nationwide EPISER study whose results were published in 2001 (Carmona et al. 2001). A total prevalence of 2.4% (95% CI 1.5% to 3.2%) for fibromyalgia was seen in the general population. Consistent with previous studies, significant gender differences were seen between females (4.2%) and males (0.2%). Prevalence rates varied between the different age groups with the peak estimate being recorded between 40 and 49 years old (4.9%) (Carmona et al. 2001).

Between 2003 and 2006, a clinical study investigating the prevalence of fibromyalgia was conducted in five European countries (France, Italy, Spain, Portugal and Germany) (Branco et al. 2010). The London Fibromyalgia Epidemiological Study Screening Questionnaires (LFESSQ-4 and LFESSQ-6) were administered to a total of 4,517 subjects; those screening positive were further evaluated for fulfilling the ACR 1990 diagnostic criteria. An overall prevalence of 4.7% (95% CI 4-5.3) was obtained based on the LFESSQ-4 positive screens as compared to 2.9% (95% CI 2.4-3.4) based on LFESSQ-6 positive screens. Again, higher prevalence was seen in females as compared to males with both screening tests. Spain had an overall prevalence of 4% (95% CI 2.8-5.2) and 2.3% (95% CI 1.4-3.2) based on LFESSQ-4 and LFESSQ-6 positive screens, respectively (Branco et al. 2010).

While the majority of prevalence studies in various countries had demonstrated prevalence estimates ranging between 1.3 and 4.4% (Lindell et al. 2000, Senna et al. 2004, Haq et al. 2005, Perrot et al. 2011, Queiroz 2013), the prevalence of fibromyalgia has been reported to be substantially lower than 1% in certain countries like Iran (0.69%) (Davatchi et al. 2008), Cuba (0.22%) (Reyes-Llerena et al. 2009) and Mexico (0.2%) (Alvarez-Nemegyei et al. 2011) and even rarely observed in China (3 cases among 6,265 subjects) (Zeng et al. 2008).

The first study that aimed to compare the prevalence of diagnosed fibromyalgia to the general population prevalence in individuals who met the ACR 2010 criteria was conducted by Vincent et al. (2013). In a retrospective approach, the authors reviewed the medical records of 3,140 subjects whereby a 1.1% of age- and sex-adjusted prevalence of diagnosed fibromyalgia was obtained. On the other hand, 6.4% of the 2,994 subjects who participated in the community survey were diagnosed with fibromyalgia (age- and sex-adjusted prevalence). Therefore, these data suggested that fibromyalgia could be underdiagnosed in the community (Vincent et al. 2013). Two surprising outcomes were reported in this study: higher prevalence of fibromyalgia in younger age groups (21-39 years: 8.45%, 40-59 years: 6.02%, and>60 years: 3.79%; P= 0.05 for sex-adjusted differences) in addition to the non-significant difference in the prevalence of fibromyalgia between males and females in the general population

(4.88% versus 7.71%, P= 0.08). Similar outcomes with respect to the non-significant difference seen in the fibromyalgia prevalence between males and females have been also reported in another study conducted in Germany (Wolfe et al. 2013). Using the ACR 2010 diagnostic criteria, the prevalence of diagnosed fibromyalgia was 2.1% (95% CI 1.6-2.7) in the general population with 2.4% (95% CI 1.5-3.2) prevalence in females and 1.8% in males (95% CI 1.1-2.6) (P= 0.372) (Wolfe et al. 2013).

1.5. Etiology

Exploring the etiological factors and the subsequent pathophysiological mechanisms underlying fibromyalgia is considered one of the most challenging objectives facing fibromyalgia investigators. Unraveling these factors would facilitate the development of adequate preventive measures in high risk populations in addition to understanding the underlying pathophysiological mechanisms. Current evidence in the literature doesn't provide definite conclusions concerning the etiology of fibromyalgia (Sommer et al. 2012), despite the extensive investigation conducted in this regard. Several factors have been proposed as being risk factors for the development of fibromyalgia such as genetic and environmental factors (Sommer et al. 2012).

1.5.1. Genetics

Several studies have investigated the evidence of any possible familial aggregation for fibromyalgia. In the study conducted by Pellegrino et al. (1989), 52% of the 50 parents and siblings of patients with primary fibromyalgia had characteristic symptoms of fibromyalgia consistent with the diagnostic criteria of Yunus et al. (1981). Accordingly, the authors concluded that fibromyalgia may be an inherited condition with an autosomal dominant mode of inheritance (Pellegrino et al. 1989). Another study that aimed to estimate the prevalence of fibromyalgia among the offspring of women with fibromyalgia showed that fibromyalgia was diagnosed in 28% of the 58 offspring included in the study (Buskila et al. 1996). After reporting the absence of any difference in psychological and familial factors in children with and without fibromyalgia, the authors attributed this familial occurrence of fibromyalgia to genetic factors (Buskila et al. 1996).

The concept of familial aggregation in fibromyalgia was emphasized in a large, controlled study which included 533 relatives of 78 probands with fibromyalgia and 272 relatives of 40 probands with rheumatoid arthritis (Arnold et al. 2004). The odd ratio (OR) measuring the odds of fibromyalgia in a relative of a proband with fibromyalgia with respect to the odds of fibromyalgia in a relative of a proband with rheumatoid arthritis was 8.5 (95% CI 2.8-26, p= 0.0002).

Genetic polymorphisms in fibromyalgia are currently perceived as being polygenetic, affecting genes in the serotoninergic, dopaminergic and catecholaminergic systems (Buskila and Sarzi-Puttini 2006). Several studies evaluated the polymorphisms affecting serotonin transporter gene (5-HTT) or the 5-HT2A receptor gene. Contradicting outcomes were reported concerning the involvement of these polymorphisms in fibromyalgia (represented in the T102C polymorphism) (Bondy et al. 1999, Offenbaecher et al. 1999, Cohen et al. 2002, Gursoy 2002, Potvin et al. 2010). In their recent meta-analysis, Lee et al. confirmed the absence of any significant association between the 5-HTTLPR polymorphism and fibromyalgia. However, the T102C polymorphism showed significant association with fibromyalgia (Lee et al. 2012).

The role of genetic polymorphisms affecting the catecholaminergic system among patients with fibromyalgia has been evaluated in four clinical studies whereby two studies confirmed the involvement of the COMT gene polymorphism in patients with fibromyalgia (Gursoy et al. 2003, Cohen et al. 2009), whereas, the other two studies reported the lack of any association between the COMT gene polymorphisms and fibromyalgia (Tander et al. 2008, Potvin et al. 2009). The COMT gene failed to reveal any association with fibromyalgia in the meta-analysis conducted by Lee et al. (2012).

Genetic polymorphisms affecting the dopaminergic system have been also investigated. Potvin et al. (2009) demonstrated the association of fibromyalgia with the dopamine-D3 (DRD3) receptor polymorphism Ser9Gly, whereas, no significant association was reported between fibromyalgia and the dopamine transporter gene polymorphism (DAT) SLC6A3 (Ablin et al. 2009).

Finally, conflicting outcomes were obtained concerning the association of monoamine oxidase (MAO) gene polymorphism in fibromyalgia; while the highly activated MAO-A allele 3 was suspected to be partly responsible for the MAOA-dependent metabolism of biological amines among patients with fibromyalgia (Gursoy et al. 2008), the MAO-A 941 genotype polymorphism was not associated to any significant role (Su et al. 2007).

1.5.2. Environmental factors

It is currently evident that the etiological factors contributing to the development of fibromyalgia extend beyond the underlying role of certain biological factors (neurophysiological and neuroendocrinological) to include psychological and environmental trigger factors. This fact led to the generation of the complex biopsychosocial model suggested by Eich et al. (2000). A broad range of environmental factors have been linked to the development of fibromyalgia such as physical and sexual abuse, physical trauma, psychological stress, certain life-style habits and infections.

1.5.2.1. Physical and sexual abuse

The influence of the various forms of abuse in fibromyalgia whether physical, sexual or emotional has been investigated in considerable number of studies. Beside the form of abuse, the different points of time at which the abuse took place whether during childhood, adolescence or adulthood have been evaluated. The initial two studies dealing with the influence of sexual and physical abuse on the appearance fibromyalgia were published in 1995 (Boisset-Pioro et al. 1995, Taylor et al. 1995). While the outcomes of the study conducted by Taylor et al. didn't reach statistical significance with respect to the increased prevalence of sexual abuse among patients with fibromyalgia, the second study conducted by Boisset-Pioro et al. demonstrated significantly higher prevalence of lifetime sexual abuse, physical abuse, combined physical and sexual abuse, and drug abuse in fibromyalgia.

These two studies were followed by an extensive number of investigations which tried to answer the possible contribution of abuse in its various forms to the development of fibromyalgia. While a majority of studies reported a positive correlation between

fibromyalgia and physical, emotional and/or sexual abuse (Carpenter et al. 1998, Castro et al. 2005, Hauser et al. 2011), other studies didn't find significant correlation (Ciccone et al. 2005, Ruiz-Perez et al. 2009). A recent meta-analysis, including 18 studies, evaluated the association between sexual and physical abuse and fibromyalgia (Hauser et al. 2011). The authors analyzed these effects separately during childhood and adulthood. A significant association was reported between fibromyalgia and sexual abuse (OR 1.95; 95% CI 1.36-2.75), physical abuse (OR 2.49; 95% CI 1.81-3.42), sexual and/or physical abuse (OR 1.78; 95% CI 1.07-2.98) and sexual as well as physical abuse (OR 2.02; 95% CI 1.06-3.87) in childhood. Similarly, a significant association was also seen between fibromyalgia and sexual abuse (OR 2.24, 95% CI 1.07-4.7) and physical abuse (OR 3.07 95% CI 1.01-9.39) in adulthood. Conversely, there was no significant association between fibromyalgia and emotional abuse neither in childhood nor adulthood.

1.5.2.2. Physical trauma

The association between fibromyalgia and physical trauma can be considered as an old postulation whereby the first study that highlighted its possible role in fibromyalgia was the clinical trial of Yunus et al. (1981). This was followed by several other studies that evaluated the viability of this possible association. In their uncontrolled follow-up study that included 176 patients diagnosed with post-traumatic fibromyalgia, Waylonis and Perkins (1994) described the onset of symptoms of fibromyalgia after motor vehicle accident in 60.7%, work injury (12.5%), surgery (7.1%), sports-related injury (5.4%) and other forms of traumatic events (14.3%).

A strong evidence for a possible association between physical trauma (cervical spine injury) and fibromyalgia was provided in two studies. In the first study, fibromyalgia was diagnosed in 21.6% following injury as compared to 1.7% of controls ($p= 0.001$) (Buskila et al. 1997). In the second study, 39% of the 136 patients with fibromyalgia reported the occurrence of physical trauma in the 6 months preceding the onset of the disease compared to 24% of the 152 controls ($p< 0.007$) (Al-Allaf et al. 2002). Conversely, other studies reported the lack of association between whiplash injury or motor vehicle

accidents with the development of widespread pain or fibromyalgia (Wynne-Jones et al. 2006, Tishler et al. 2011).

1.5.2.3. Stress

Beside the association between fibromyalgia and psychological stress caused by early-life trauma such as parental loss or abuse (Gupta and Silman 2004), other studies have investigated the influence of other forms of psychological stress such as economic problems, conflict with parents and occupational stress. In the study conducted by Anderberg et al. (2000), higher proportion of patients with fibromyalgia reported the occurrence of very negative life events (51%) compared to controls (28%) during childhood or adolescence ($p< 0.05$).

In another cohort study, the authors aimed to examine the association between occupational stress and incidence of newly diagnosed fibromyalgia (Kivimaki et al. 2004). The OR for workplace bullying was 4.1 (95% CI 2-9.6), high workload (OR 2.1, 95% CI 1.2-3.9) and low decision scope (OR 2.1, 95% CI 1.1-4). The interaction between stress and characteristic symptoms of fibromyalgia has been examined in a recent cross-sectional study (Malin and Littlejohn 2013). Significant correlation has been established between perceived stress and characteristic features of fibromyalgia such as pain ($p< 0.05$), sleep change, fatigue and cognitive dysfunction (all $p< 0.001$) suggesting a major role for stress in modulating characteristic symptoms of fibromyalgia (Malin and Littlejohn 2013).

1.5.2.4. Other factors

The overlap of considerable number of manifestations between fibromyalgia and certain viral or atypical infections led to suspecting a potential role of infections in provoking fibromyalgia. Several infections such as hepatitis C or B, HIV and Lyme disease have been linked to the development of fibromyalgia (Buskila et al. 2008). Even some evidence exists for a possible role of vaccinations in triggering fibromyalgia syndrome (Buskila et al. 2008). The prevalence of fibromyalgia among patients suffering from certain infections has been shown to be relatively elevated as compared to the prevalence in the general population. For instance, it has been diagnosed in 16% of

patients with HCV (Buskila et al. 1997), 26% of patients with HBV (Adak et al. 2005), 29% of patients with HIV (Buskila et al. 1990) and 8% among patients with Lyme disease (Dinerman and Steere 1992).

Fibromyalgia has been also shown to be significantly associated to the different types of allergies and other life-style habits such as lack of physical activity, increased body mass index (BMI) (Mork et al. 2010) and smoking (Choi et al. 2010).

1.6. Pathophysiology

The precise pathophysiological mechanisms underlying fibromyalgia are not yet fully understood. The complexity of this syndrome represented in the broad symptomatologic spectrum requires the acknowledgment of these pathophysiological mechanisms from a multidisciplinary perspective rather than adopting a reductionist approach (Abeles et al. 2007). Several hypotheses have been suggested for the potential mechanisms underlying fibromyalgia such as: central nervous system abnormalities, autonomic nervous system dysfunction and alterations in the neuroendocrine function, among the most relevant.

1.6.1. Central nervous system abnormalities

Central nervous system abnormalities in fibromyalgia have been suggested to be responsible for the appearance of the complex spectrum of manifestations. Both structural and functional abnormalities have been reported. Differences in brain responses, at rest or following painful stimuli, between patients with fibromyalgia and healthy subjects have been well documented in neuroimaging studies (Gracely and Ambrose 2011). Reduced neural activity among patients with fibromyalgia has been demonstrated through decreased regional cerebral blood flow in bilateral thalamus, caudate nucleus, inferior pontinetegmentum and near the right lentiform nucleus as compared to control subjects (Mountz et al. 1995, Bradley et al. 1999, Kwiatek et al. 2000).The reduced thalamic activity, which could represent an inhibition in response to prolonged excitatory activity, has also been found in other chronic pain conditions (Gracely and Ambrose 2011). Thus, it could be a signature of chronic pain in general and may not be unique to fibromyalgia (Abeles et al. 2007). In addition to the functional

differences, other studies have demonstrated that fibromyalgia is also associated to structural changes in the brain whereby greater age-associated loss in the volume of the grey matter volume was reported, with each year of fibromyalgia being equivalent to 9.5 times the loss in normal aging (Kuchinad et al. 2007). The functional significance of the grey matter atrophy is hypothesized to be related to impaired endogenous pain inhibition and cognitive function deficit (Gracely and Ambrose 2011).

The comparison of the brain responses to painful peripheral stimuli, on the functional magnetic resonance imaging, demonstrated an increased cerebral flow at much lower thresholds in patients with fibromyalgia as compared to healthy controls (Gracely et al. 2002). Interestingly enough, the pressure in this study was applied to the thumb nail beds which are devoid of muscle, addressing the hypothesis that tenderness could be mediated via deep tissue receptors in both muscular and non-muscular tissue. Despite the discrepancies in the outcomes of neuroimaging studies in fibromyalgia (Abeles et al. 2007), these studies played a crucial role in the appearance of the subsequent explanatory hypotheses concerning the involvement of central nervous system processes at the level of the brain and the spinal cord in fibromyalgia. Among these mechanisms are the central nervous system sensitization, dysregulation of descending inhibitory pain pathways and alterations in the neurotransmitters' function.

1.6.2. Central sensitization

Central sensitization is defined as an amplification of neural signaling within the central nervous system that elicits pain hypersensitivity (Woolf 2011). It is characterized by prolonged and reversible increase in the excitability and synaptic efficacy of neurons in central nociceptive pathways. It is manifested as reduction in threshold (allodynia), increased and prolonged responsiveness to noxious stimuli (hyperalgesia), and a receptive field expansion that enables the sensory input from non-injured tissue (secondary hyperalgesia) (Woolf 2011).

The long standing sensitization of primary sensory and dorsal horn wide-dynamic-range (WDR) neurons can be considered an expression of neural plasticity (Nielsen and Henriksson 2007). This neural plasticity is associated with longstanding bombardment

of neurons in the spinal cord leading to the maintenance of central sensitization (Nielsen and Henriksson 2007) through the activation of the N-methyl-D-aspartic acid (NMDA) receptors which are found on the postsynaptic membrane in the dorsal horn of the spinal cord (Abeles et al. 2007). The activation of the NMDA receptors causes additional release of neuropeptides such as substance P leading to the spread of pain. Accordingly, the repetitive stimulation of the dorsal horn neurons from C nociceptive afferent neurons gives rise to gradually increasing pain responses. This phenomenon of slow temporal summation is termed “windup” (Staud et al. 2001).

The evidence for the presence of central sensitization in fibromyalgia began with the demonstration of decreased pain thresholds at tender and non-tender point sites as compared to control subjects (Tunks et al. 1988). Quantitative methods revealed decreased both mechanical (Wolfe et al. 1990, Maquet et al. 2004) and thermal pain thresholds among patients with fibromyalgia (Gibson et al. 1994). The description of pain at tender points as secondary hyperalgesia, indicating central sensitization, was suggested after reporting the NMDA receptors involvement in pain perception in fibromyalgia. In the study conducted by Sörensen et al. (1995), the intravenous injection of ketamine, an NMDA receptor antagonist, led to significant reduction in pain intensity, decreased tenderness and increased endurance among patients with fibromyalgia. In another study, ketamine use was also associated to attenuation of the mechanisms involved in referred pain, temporal summation, muscular hyperalgesia and muscle pain in fibromyalgia (Graven-Nielsen et al. 2000). The authorsof this last study concluded the presence of a link between central hyperexcitability and the mechanisms that facilitate referred pain and temporal summation in a subgroup of patients with fibromyalgia (Graven-Nielsen et al. 2000).

Evidence for the abnormal processing of the input to central nociceptive pathways was investigated in various studies conducted by Staud et al. (2001, 2003, 2005). Patients with fibromyalgia were shown to display exceedingly higher levels of temporal summation and longer lasting after sensations (Staud et al. 2001, Staud et al. 2003). Given the established role of NMDA and substance P receptors in eliciting windup and the enhanced temporal summation in fibromyalgia, Staud et al. (2005) conducted a

double-blind, placebo-controlled, cross over study to investigate the effects of NMDA receptor antagonist dextromethorphan on windup in fibromyalgia. It was shown that the difference in the extent of windup reduction by dextromethorphan between patients with fibromyalgia and controls didn't reach statistical significance. Thus, the authors claimed that other mechanisms need to be considered for the widespread pain in fibromyalgia (Staud et al. 2005).

Another pathway that could possibly contribute to central sensitization is the activation of glial cells that mediate their effects through the release of neuroactive substances and proinflammatory cytokines in addition to their upregulation, as well as the release of substance P and excitatory neurotransmitters in the spinal cord (Abeles et al. 2007). Thus, they are believed to play substantial role in the modulation of pain signaling in the central nervous system. In fibromyalgia, a study was conducted by Kadetoff et al. (2012) demonstrated the presence of elevated cerebrospinal fluid and serum levels of the proinflammatory cytokine interleukin-8.

In conclusion, a consensus exists concerning an underlying role of central sensitization in the generation of chronic pain in fibromyalgia. The reduction of pain secondary to pregabalin and duloxetine in fibromyalgia may indicate a reduction in central sensitization among these patients (Woolf 2011).

1.6.2.1. Dysregulation of descending inhibitory pain pathways

In addition to the amplification of pain resulting from central sensitization, another finding that could as well contribute to the amplification of pain is the alteration in the normal pain processing through the dysfunction of the descending inhibitory pain pathways. Under normal conditions, there are regions in the central nervous system that participate in pain modulation by either inhibiting or facilitating the transmission of the nociceptive input at the level of the dorsal horn; these modulatory effects are largely mediated by descending monoaminergic pathways via serotonin, norepinephrine or dopamine (Kwon et al. 2013). Diminished activity of descending inhibitory pathways, decreased synthesis of inhibitory neurotransmitters (GABA and glycine), loss of inhibitory interneurons, in addition to the diminished activity of serotonin and

norepinephrine are thought to contribute to persistent and chronic pain mechanisms (Kwon et al. 2013).

Another form of inhibition in the descending inhibitory pain pathways includes the “diffuse noxious inhibitory controls (DNIC)” (Gracely and Ambrose 2011). The DNIC are characterized by widespread inhibitory effects occurring throughout the body, hence, termed “diffuse”. DNIC are triggered by a sustained nociceptive input which provokes a tonic level of inhibition under normal conditions. The loss of this inhibition can be associated to unusual widespread pain and tenderness (Gracely and Ambrose 2011). In a study conducted by Staud et al. (Staud et al. 2003), it was demonstrated that the DNIC attenuated the thermal windup pain in normal males but failed to do so in normal females or in patients with fibromyalgia suggesting that DNIC effects are gender-specific with women generally lacking this pain-inhibitory mechanism. However, results concerning the gender-specific effect of DNIC are controversial. In a systematic review conducted by Popescu et al. (2010), it was reported that the majority of studies evaluating patient-reported pain scores as an outcome showed significantly more efficient DNIC in males, whereas studies evaluating pain thresholds and nociceptive flexion reflex indicated non-significant differences in the mean DNIC effects between males and females. Hence, the authors concluded that gender differences in the DNIC vary depending on the methodological procedures being utilized.

Finally, evidence for abnormalities in the descending pain inhibition pathways in fibromyalgia has been also established using functional magnetic resonance imaging studies whereby mechanical stimuli among patients with fibromyalgia were not associated with any activity at the level of the rostral anterior cingulate which is the region involved in pain inhibition. On the other hand, successful treatment in fibromyalgia was linked to increased brain activity in the regions involved in the descending inhibitory pathways (Gracely and Ambrose 2011).

1.6.2.2. Alterations in the neurotransmitters’ function

Serotonin, 5-hydroxytryptamine, is a neurotransmitter produced by neurons in the brain stem. Serotonin is widely distributed as serotonergic neurons extend from the posterior

raphe nucleus to the medulla and spinal cord and they make connections throughout the cortex, limbic system, and thalamus (Gupta and Silman 2004, Abeles et al. 2007). Serotonin exerts inhibitory effects on several pain pathways via the suppression of substance P production (a nociceptive neurotransmitter).

In fibromyalgia, the outcomes of clinical studies investigating the serum levels of serotonin yielded conflicting data. Two clinical studies reported that serotonin levels were significantly lower among patients with fibromyalgia as compared to healthy controls (Yunus et al. 1992, Stratz et al. 1993). On the other hand, despite the significantly lower level of plasma serotonin among patients with fibromyalgia reported by Wolfe et al. (1997), association between serotonin and tender point count was of opposite direction as compared to previous reports (r= 0.563).

Concerning serotonin levels in the central nervous system, Russell et al. (1992) reported Decreased cerebrospinal fluid levels of biogenic amines metabolites of serotonin, norepinephrine and dopamine among patients with fibromyalgia as compared to patients with rheumatoid arthritis and healthy controls has been also reported (1992).

Substance P, a neuropeptide neurotransmitter, plays an important role in the process of nociception at the level of the dorsal horn in the spinal cord. Substance P exerts an excitatory action by alerting the spinal cord to incoming nociceptive signals (Russell 1998). In fibromyalgia, several studies have reported elevated cerebrospinal levels of substance P (Russell et al. 1994, Bradley 1996).

Dopamine, a catecholamine neurotransmitter, is mainly found in the corpus striatum which receives the major input from substantia negra. An evidence for a certain role of dopamine in modulating pain perception and natural analgesia within supraspinalregions has been suggested (Wood 2008). In fibromyalgia, Wood et al. have conducted several investigations concerning its potential underlying role. In their pilot study (Wood et al. 2007), the assessment of presynaptic dopamine activity in 6 patients with fibromyalgia revealed disrupted uptake in several brain regions. In their second clinical study (Wood et al. 2009), fibromyalgia was linked to altered brain morphometry

represented in reductions in the gray matter density within brain regions responsible for pain perception, cognitive function and stress reactivity.

1.6.3. Autonomic nervous system dysfunction

The abnormal functioning of the autonomic nervous system is believed to contribute to aggravated pain and other clinical manifestations in fibromyalgia through alterations in the physiologic processes involved in stress management and pain inhibition. Abnormalities include decreased microcirculatory vasoconstriction, heart rate variability, hypotension and circadian rhythm abnormalities (Bradley 2008).

Altered activity of the sympathetic nervous system in fibromyalgia has been documented in several studies. In the study conducted by Vaerøy et al. (1989), the vasoconstrictory responses to auditory or cold water stimulations were significantly lower among patients with fibromyalgia as compared to healthy controls. The same group later reported increased baseline skin blood flow and less vasoconstriction during acoustic stimulation and cold pressor tests, suggesting increased cholinergic activity or decreased sympathetic nervous system activity in fibromyalgia (Qiao et al. 1991). Concerning the tilt table testing among patients with fibromyalgia, abnormal drop in blood pressure was seen in 60% of patients with fibromyalgia and none of the controls (Bou-Holaigah et al. 1997). In another study that examined the response to tilt table test in fibromyalgia, only 19% of the patients with fibromyalgia were positive as compared to 9% of the controls (Clauw 1996).

Heart rate variability has been also shown to be altered among patients with fibromyalgia indicating an abnormality in the autonomic nervous system in this group of patients. While in standing position, heart rate variability at the 0.05 Hz to 0.150 Hz frequency domain was significantly lower among patients with fibromyalgia as compared to control subjects (Martinez-Lavin et al. 1997). In another study conducted by Stein et al. (2004), it was found that the decreased heart rate variability in fibromyalgia was sex-dependent, whereby significantly greater decrease in heart rate variability was seen among women than in men with fibromyalgia. Using heart rate variability analysis to determine the accumulated 24-hour cardiovascular autonomic

modulation and its circadian variations in fibromyalgia, Martinez-Lavin et al. (1998) showed that heart rate variability was diminished among patients with fibromyalgia as compared to matched controls, linking this finding to the increased nocturnal predominance of low-frequency band oscillations consistent with an exaggerated sympathetic modulation of the sinus node.

Dysautonomia in fibromyalgia is characterized by a paradoxical behavior. It is characterized by unrelenting sympathetic hyperactivity at rest and hypo-reactivity of the sympathetic response is documented todifferent stressors such as exercise, standing or cold exposure (Martinez-Lavin and Hermosillo 2000, Furlan et al. 2005, da Cunha Ribeiro et al. 2011). Accordingly, it has been postulated that this concurrent hyper-hypo activity can explain the occurrence of several multisystem manifestations of fibromyalgia. While persistent sympathetic hyperactivity is thought to facilitate insomnia, irritable bowel, anxiety and dryness in the eyes and mouth, sympathetic hyporeactivity may cause fatigue (Martinez-Lavin and Vargas 2009).

1.6.4. Alterations in the neuroendocrine function

The symptomatologic overlap between fibromyalgia and certain endocrine deficiencies (such as cortisol, growth hormone and thyroid hormone) raised the possibility of a possible underlying role of the various hypothalamic-pituitary-peripheral gland axes in fibromyalgia (Adler et al. 2002). In addition, the established role of stress in its various forms (physical, psychological and emotional) in the development of fibromyalgia also constitutes another indication for the possible involvement of abnormal neuroendocrine function in fibromyalgia.

The pulsatile secretion of specific pituitary hormones is regulated by the hypothalamic hormones. The pituitary hormones act on the target peripheral glands to stimulate hormone secretion. Various stressors such as emotional stress, exercise, hypoglycemia, hypotension, infections and pain influence the frequency of pituitary pulsatile secretions (Martini 2010). Several studies have investigated the potential role of hypothalamic-pituitary-adrenal (HPA), hypothalamic-growth hormone-insulin-like

growth factor-1 and hypothalamic-pituitary-thyroid axes in the pathogenesis of fibromyalgia.

1.6.4.1. Hypothalamic-pituitary-adrenal axis

The HPA axis is considered as the primary endocrine stress axis in humans. Clinical studies have demonstrated variable outcomes concerning the difference in cortisol levels between patients with fibromyalgia and healthy controls. The withdrawal of steroid therapy, which is usually associated with low levels of CRH, ACTH and cortisol, is linked to symptoms similar to those described in fibromyalgia such as myalgias, fatigue, gastrointestinal complaints and impaired cognitive function (Adler et al. 2002).

In fibromyalgia, an evidence for underactive HPA axis is suggested as several studies have found reduced plasma cortisol or decreased 24-hour urinary-free cortisol excretion (Crofford et al. 1994, Lentjes et al. 1997, Gupta and Silman 2004, Izquierdo Alvarez et al. 2009). In a meta-analysis evaluating the HPA axis function in three functional somatic disorders (fibromyalgia, chronic fatigue syndrome and irritable bowel syndrome), the authors found a generally lower basal cortisol levels among patients with functional somatic disorders as compared to placebo, however, findings didn't reach statistical significance (95% CI -0.17 to 0.04, p = 0.241) (Tak et al. 2011). When basal cortisol levels were evaluated separately in each of the three disorders, statistically significant basal hypocortisolism was observed in CFS subjects compared to controls (95% CI -0.28 to 0.00, p = 0.047), but not in fibromyalgia or irritable bowel syndrome (Tak et al. 2011).

1.6.4.2. Hypothalamic-growth hormone- insulin-like growth factor-1

The symptoms of growth hormone deficiency (GH), which include impaired cognition, fatigue, muscle weakness and decreased exercise tolerance, have raised the suspicion of a possible underlying GH deficiency in fibromyalgia. Several clinical studies comparing the difference in basal GH and IGF1 levels between patients with fibromyalgia and healthy controls reported lower levels in the former group especially during sleep periods (Gupta and Silman 2004). GH is largely secreted during the third and fourth stage of non-rapid eye movement sleep (Gupta and Silman 2004);

consequently, it was postulated that the abnormal GH levels in fibromyalgia are a consequence of the altered sleep in fibromyalgia. Accordingly, Bennett et al. (1997) reported the presence of a subgroup of patients with fibromyalgia with initial normal levels of IGF-1 who experienced a rapid decline over 1 to 2 years. However, several factors other than sleep disturbances have been linked to decreased GH levels in fibromyalgia including obesity, depression, physical fitness and estrogen use (Jones et al. 2007).

Despite the benefit reported with GH therapy over 9-month period in fibromyalgia (Bennett et al. 1998), most patients with fibromyalgia who present low IGF-1 levels have been reported to have normal pituitary GH response to insulin tolerance test and growth hormone releasing hormone/arginine suggesting that the changes affecting this axis are most likely hypothalamic in origin (Jones et al. 2007).

1.6.4.3. Hypothalamic-pituitary-thyroid axis

Among the various clinical manifestations commonly seen in hypothyroidism, it is noteworthy the partial overlap of some of these manifestations such as fatigue, impaired memory, depression and muscle weakness and cramps with fibromyalgia (Roberts and Ladenson 2004). Although the basal levels of total and free tetraiodothyronine, thyroid-stimulating hormones (TSH) and thyroid hormones binding globulin have been found to be normal in fibromyalgia (Neeck and Riedel 1992, Neeck and Riedel 1999, Rodriguez-Espinosa et al. 2006), a reduced increase in TSH following the administration of thyrotropin releasing hormone (TRH) has been reported (Neeck and Riedel 1992).

In the study conducted by Riedel et al. (Riedel et al. 1998), blunted responses of the free T3 and TSH have been found following the injection of TRH. The authors attributed this observation to the possible augmented secretion of somatostatin acting on pituitary thyrotrophswhich thus alter the responsiveness of the pituitary thyrotrophs to TRH (Riedel et al. 1998).

1.7. Symptomatology

Fibromyalgia is characterized by a complex nature and a wide array of signs and symptoms. Chronic generalized musculoskeletal pain represents the cardinal symptom that defines fibromyalgia (Smith et al. 2011). In addition to widespread pain, chronic fatigue and sleep disturbances complement the hallmark triad of the core symptoms of fibromyalgia (Arnold et al. 2011). Wider range of manifestations can be also seen among patients with fibromyalgia including morning stiffness, headaches, balance problems, cognitive dysfunction (forgetfulness and poor concentration), sexual dysfunction, dysesthesia and psychological distress (anxiety and depression) (Bennett 2009).

As stated above, the chronic widespread pain of fibromyalgia is characterized by a bilateral nature affecting the left and right side of the body in addition to the regions below and above the waist. Axial skeletal pain is also present and generalized pain is usually present over a period that exceeds 3 months (Smith et al. 2011). Generalized pain of fibromyalgia is perceived as originating in the muscles or deep in bones and common sites include lower back, neck, shoulders, arms, knees, hips, thighs, legs feet and anterior chest (Cassisi et al. 2008). Pain in fibromyalgia is characterized by allodynia, hyperalgesia, persistence, pronounced summation effects, hyperpathia in the skin and tenderness on examination and patients describe their pain as any combination of burning, searing, tingling, shooting, stabbing, deep aching, sharp and/or feeling bruised all over (Jain et al. 2003). However, pain in fibromyalgia is also characterized by “non-anatomical” distributions as it doesn’t follow any definite or nerve root distributions and it is not necessarily confined to the eighteen classical tender points. It is also characterized by a non-specific nature as it can even originate in unexpected places at unexpected times and can change day to day or even hour to hour (Jain et al. 2003).

Several modulating factors have been shown to affect the severity of pain in fibromyalgia. These factors include noise, fatigue, stress, physical activity, anxiety, humidity, warmth, cold, poor sleep and weather change (Wolfe et al. 1990). Additionally, chronobiological factors have been also shown to influence the severity of fibromyalgia

symptoms. Patients reported variable severity of symptoms throughout the day, month or even year. Daily evaluation demonstrated that the worst times for patients with fibromyalgia are in the morning, later half of the afternoon and evening leaving the patient with a narrow interval (10:00 to 14:00 h) during which greater tolerance to discomfort or less pain is experienced. Monthly evaluations of symptoms during which patients with fibromyalgia claimed most pain, worst mood and least restful sleep extended from November to March, whereas, fewest symptoms were seen from May to August (Moldofsky 1994).

Fatigue is one of the most commonly reported manifestations in fibromyalgia. Patients with fibromyalgia frequently use the expression “I am always tired” to describe their fatigue which has been also described with exhaustion, tiredness, lack of energy, and a global feeling of general weakness. In a study conducted to assess the prevalence of fatigue in rheumatic diseases (Wolfe et al. 1996), fatigue was reported by 88-98% of patients with rheumatic diseases and was shown to be clinically relevant in 76% of patients with fibromyalgia. In an internet survey conducted in Germany, fatigue was reported by 99.1% of the 699 respondents, occupying the third rank in prevalence following muscle pain (99.7%) and low back pain (99.6%) (Hauser et al. 2008). Comparable outcomes were obtained in another internet survey conducted in the United States including 2,569 participants, whereby the highest rated symptoms in terms of intensity included morning stiffness followed by fatigue (Bennett et al. 2007). In an attempt to establish a core domain set for assessment of fibromyalgia in clinical practice and clinical trials, the OMERACT 9 (Outcome Measures in Rheumatology Clinical Trials) workshop demonstrated that more than 70% of patients with fibromyalgia considered that pain, tenderness, fatigue, patient global, multidimensional function and sleep disturbance domains should be evaluated in all fibromyalgia clinical trials (Mease et al. 2009).

The intensity of fatigue among patients with fibromyalgia is affected by several factors. It is generally worse in the morning, as such, patients wake up feeling more exhausted as compared to when they go to bed (Cassisi et al. 2008). Although depression and non-restorative sleep are considered as the most obvious contributors to fatigue in

fibromyalgia, antidepressant therapy is often associated to modest improvement in fatigue (Bennett 2009). Similarly, improvements in sleep, following the administration of sodium oxybate among patients with fibromyalgia, have been linked to slight improvement in fatigue (Russell et al. 2009).

Sleep disturbances are frequently seen among patients with fibromyalgia who report sleep disturbances that include difficulties with sleep initiation and maintenance which is referred to as non-restorative sleep (NRS) (Bennett 2009). Consequently, patients with fibromyalgia experience significant day time impairment with increased fatigue and tiredness (Spaeth et al. 2011). Patients with fibromyalgia describe their nightly sleep to be light and unrefreshing irrespective of its duration with discomfort in the lower extremities represented in restlessness, uncontrollable kicking, and involuntary leg movements before and during sleep (Moldofsky 2008). Therefore, the evaluation of disturbed sleep in patients with fibromyalgia requires an initial evaluation for the presence of primary sleep problems. The elevated comorbidity of sleep disorders in fibromyalgia such restless leg syndrome (64%) is linked to more sleep disturbances and pronounced daytime sleepiness (Bennett 2009).

Polysomnographic recordings in fibromyalgia indicated abnormal findings in the electroencephalogram (EEG). Abnormally elevated amounts of alpha EEG during non-rapid eye movement sleep have been documented and the analyses of EEG in fibromyalgia indicate three varieties of alpha EEG sleep pattern: phasic (50% of patients vs. 7% healthy controls), tonic (20% of patients vs. 9% healthy controls) and low alpha (30% of patients vs. 84% health controls) (Moldofsky 2008). Thus, the intrusion of alpha waves on delta rhythm (loss of deeper phases of sleep) along with the reduced portion of slow-wave sleep and rapid eye movement sleep are thought to be responsible for the NRS in fibromyalgia (Spaeth et al. 2011).

Stiffness is another important manifestation that is frequently encountered among patients with fibromyalgia. It is not limited to articular regions but rather it is generalized (global stiffness) with maximum incidence occurring upon awakening and in the evening (Cassisi et al. 2008). Patients perceive stiffness among the most troublesome

manifestations in fibromyalgia as it classified as the first in terms of symptoms intensity in the survey conducted by Bennett et al. (2007) and it was the fourth most prevalent manifestation (97.6%) as reported in the survey conducted by Hauser et al. (2008).

Patients with fibromyalgia frequently suffer from cognitive dysfunction which includes difficulties with short-term memory, concentration, logical analysis, dual tasking, motivation and verbal fluency (Bennett 2009). Compared to age- and education-matched controls, patients with fibromyalgia performed more poorly on most cognitive measures and their performance was not different from health adults 20 years older (Park et al. 2001). Comorbid depression, anxiety, insomnia and fatigue are thought to play a prominent role in affecting the cognitive function in fibromyalgia; however, they don't entirely explain the mental complaints experienced by patients with fibromyalgia (Bertolucci and de Oliveira 2013). In a systematic review conducted by Gelonch et al. (2013), it was reported that a consensus exists concerning the direct correlation between the degree of pain and cognitive dysfunction in fibromyalgia. On the other hand, there is no consensus with respect to the influence of depression and anxiety on cognitive impairment in fibromyalgia (Gelonch et al. 2013).

Another debilitating manifestation of fibromyalgia is the high frequency of psychological disturbances such as depression, anxiety disorders, panic attacks and post-traumatic stress disorder (Buskila and Cohen 2007). Psychological distress is believed to perpetuate chronic pain which in turn may further aggravate psychological disturbances (Cassisi et al. 2008). In the study conducted by Arnold et al. (2006), the co-occurrence OR for specific psychological disorders in fibromyalgia compared to subjects without fibromyalgia (controls and rheumatoid arthritis patients) were as follows: bipolar disorder (OR= 153, 95% CI 26-902; $p< 0.001$), major depressive disorder (OR= 2.7, 95% CI 1.2-6; $p= 0.013$) and anxiety disorder (OR= 6.7, 95% CI 2.3-20; $p<0.001$). Major depressive disorder is seen in around 30% of patients with fibromyalgia at the time of diagnosis whereas lifetime prevalence proportions of depression and anxiety reach an estimate of 74%, and 60%, respectively (Buskila and Cohen 2007). In the study conducted by Giesecke et al. (2003), the authors assumed that patients with fibromyalgia diagnosed by the ACR criteria do not constitute a homogenous group, but rather several factors

could contribute to the varying clinical manifestations, among which are the psychological factors. It was demonstrated that the coexistence of high levels of anxiety and depression is linked to lower ability of pain control and suprathreshold pain-sensitivity levels (Giesecke et al. 2003).

1.8. Comorbidities

Fibromyalgia is currently classified under the group of syndromes known as central sensitivity disorders (Yunus 2007), which encompass overlapping conditions that share common features of central sensitization characterized by abnormal and intense enhancement of pain perception (manifested as hyperalgesia, allodynia and receptive field expansion) (Yunus 2007). In addition to fibromyalgia, central sensitivity disorders comprise multitude of disorders such as chronic fatigue syndrome, irritable bowel syndrome, temporomandibular joint dysfunction and tension headache, among others (Woolf 2011). Accordingly, patients with fibromyalgia frequently suffer from a wide array of comorbid conditions. In a large cohort retrospective study including 2,595 cases of fibromyalgia identified between 1997 and 2002, subjects seemed to be 2 to 7 times more prone to suffer from headaches, irritable bowel syndrome, chronic fatigue syndrome, depression, anxiety, systemic lupus erythematosus, and rheumatoid arthritis (Weir et al. 2006). Other studies investigating the prevalence of comorbid conditions among patients of fibromyalgia have documented the occurrence of migraine headache (35%), systemic lupus erythematosus (1-65%), chronic fatigue syndrome (70%) and rheumatoid arthritis (17%) (Weir et al. 2006).

Conversely, increased prevalence of fibromyalgia is reported among patients with chronic fatigue syndrome (55%), irritable bowel syndrome (40.7%), primary headaches (26.3%), temporomandibular disorder (23.7%), vulvar vestibular syndrome (23.4%), interstitial cystitis (15.4%) and gulf war syndrome (17.6%) (Yunus 2012).

1.9. Therapeutic management

The underlying complexity of fibromyalgia with its wide array of manifestations complicate the attempts aiming to formulate adequate therapeutic management plan and make this syndrome a “not easy to treat” condition. Different therapeutic options are

currently available for the treatment of patients with fibromyalgia including pharmacologic and non-pharmacologic measures in addition to complementary and alternative medicine options.

1.9.1. Pharmacologic Therapy

The current clinical practice in fibromyalgia is limited by the complex nature of the disease. This is reflected by the lack of a single agent able to control all the symptoms of the disease and in the limited number of available pharmacotherapies that are approved by the drug regulatory authorities for the treatment of fibromyalgia. While only three drugs have been approved by the US Food and Drug Administration (FDA), pregabalin in 2007, duloxetine in 2008, and milnacipran in 2009, the European Medicines Agency (EMA) has not granted the approval for any drug so far. The currently available pharmacologic options for the treatment of fibromyalgia are discussed below.

1.9.1.1. Antidepressants

Besides their role in the management of depression and anxiety disorders, antidepressants are effective in the treatment of chronic pain modifying both the central and peripheral sites involved in pain transmission and perception (Table 1) (Sawynok et al. 2001, Verdu et al. 2008). Several clinical studies have investigated the efficacy of the various antidepressant classes in the treatment of fibromyalgia, including tricyclic antidepressants (TCA), serotonin and norepinephrine reuptake inhibitors (SNRI), and selective serotonin reuptake inhibitors (SSRI) (Table 2).

Table 1 Pharmacologic mode of actions of antidepressants in relation with persistent pain signaling*

Mechanism of Action	Site of action	TCA	SNRI	SRI
Reuptake inhibition of Monoamine	Serotonin	+	+	+
	Noradrenaline	+	+	–
Receptor Antagonism	α-Adrenergic	+	–	–
	NMDA	+	(+) milnacipran	–
Blocker or activator of ion channels	Sodium channel blocker	+	(+) venlafaxine – duloxetine	(+) fluoxetine
	Calcium channel blocker	+	?	(+) citalopram fluoxetine
	Potassium channel activator	+	?	–
$GABA_B$receptor	Increase of receptor function	+ amitriptyline desipramine	?	+ fluoxetine
Opioid receptor binding/opioid-mediated effect	μ- and δ-Opioid receptor	(+)	(+) venlafaxine	(+) paroxetine

Abbreviations: SNRI= serotonin and norepinephrine reuptake inhibitor; SRI= selective serotonin reuptake inhibitor; TCA= tricyclic antidepressant; + indicate mechanism of action documented in vitro and/or in vivo; (+) indicates mechanism of action documented in vitro and/or in vivo at high concentration;– indicates no known mechanism of action; ? indicates not investigated/not known

*: Adapted from Verdu et al. (2008)

Table 2 Effect sizes of the different classes of antidepressants on different fibromyalgia symptoms*

Outcome	Effect Size (95% CI)**		
	TCA	*SSRI*	*SNRI*
Pain	-1.64 (-2.57 to -0.71)	-0.39 (-0.77 to -0.01)	-0.36 (-0.46 to -0.25)
Fatigue	-1.12 (-1.87 to -0.38)	-0.17 (-0.47 to 0.12)	-0.08 (-0.20 to 0.05)
Sleep	-1.84 (-2.62 to -1.06)	-0.23 (-0.56 to 0.10)	-0.31 (-0.47 to -0.14)
Depressed mood	-0.60 (-4.53 to 3.33)	-0.37 (-0.66 to -0.07)	-0.26 (-0.42 to -0.10)
HRQOL	-0.31 (-0.60 to -0.01)	-0.41 (-0.78 to -0.05)	-0.31 (-0.44 to -0.17)

Abbreviations: CI= Confidence Interval; TCA= Tricyclic antidepressant; SSRI= Selective serotonin reuptake inhibitor; SNRI= Selective serotonin norepinephrin reuptake inhibitor; HRQOL= Health-related quality of life.

*: Adapted from Häuser et al. (2009), **: Small = 0.2-0.49; Medium = 0.5-0.79; Large ≥0.8

1.9.1.1.1. Tricyclic antidepressants

Tricyclic antidepressants are one of the oldest antidepressant classes. Their clinical use has not been restricted to depression, as they are widely used in different chronic pain conditions including neuropathic pain, headache, low back pain and fibromyalgia (Verdu et al. 2008). In a recent meta-analysis that evaluated the efficacy and safety of antidepressants in the treatment of fibromyalgia, either TCAs, SSRIs, and SNRIs were associated with significant improvement of improving pain, sleep, fatigue, depression and health-related quality of life; however the TCAs had the largest effect sizes in improving pain and sleep disturbances as compared to the other classes (Hauser et al. 2009, Hauser et al. 2012). In another meta-analysis comparing amitriptyline with duloxetine and milnacipran, it was found that amitriptyline was superior in reducing pain, sleep disturbances, fatigue and health-related quality of life; however, and due to the methodological limitations of the trials (small number of patients and short-termed studies), the authors concluded that amitriptyline could not be regarded as the gold-standard in the treatment of fibromyalgia (Hauser et al. 2011). This fact confirms the need for well-designed, long-term clinical trials able to evaluate the actual efficacy of amitriptyline in fibromyalgia. The doses of TCAs showing efficacy in fibromyalgia are lower than those recommended for the treatment of major depressive disorder, which is not the case for other antidepressant classes; this is probably due to their multiple mechanisms of action, both at central and peripheral sites, involved in their capacity to counteract pain-generating mechanisms (Verdu et al. 2008).

1.9.1.1.2. Selective Serotonin Norepinephrine Reuptake Inhibitors

To date, the three SNRIs that have been tried in the treatment of fibromyalgia are venlafaxine, duloxetine and milnacipran. The latter blocks with similar affinity the reuptake of serotonin and noradrenaline whereas duloxetine shows a 10-fold selectivity and venlafaxine a 30-fold selectivity for serotonin (Stahl et al. 2005). In spite of the fact that venlafaxine was the first SNRI to be studied in fibromyalgia, it has not been extensively investigated in this indication as it has been shown to be effective in alleviating pain and depressive symptoms only in two small uncontrolled trials (Dwight et al. 1998, Sayar et al. 2003). Duloxetine 60-120 mg/day and milnacipran 100-200

mg/day have been shown to significantly reduce pain and depressive symptoms and to improve quality of life; while duloxetine therapy lead to significant improvement in sleep disturbances, it had non-significant effect on fatigue that was, on the other hand, significantly improved by milnacipran (Hauser et al. 2010, Hauser et al. 2011). Duloxetine has been shown to reduce pain and other symptoms of fibromyalgia both in patients with or without major depressive disorder (Arnold et al. 2009). In contrast to the short-term studies investigating the effect of TCAs in fibromyalgia, duloxetine and milnacipran have been studied in 1-year follow-up studies, where their efficacy has been shown to be preserved over the entire period for both antidepressants with a good tolerability and safety profile (Chappell et al. 2009, Branco et al. 2011).

1.9.1.1.3. Other antidepressants

Selective serotonin reuptake inhibitors are not as effective as the other antidepressants in the treatment of fibromyalgia. In the meta-analysis performed by Häuser and coworkers (2012) although the effects of SSRIs on pain, sleep, fatigue depression and quality of life were statistically significant, their effects sizes were found to be small or non-substantial. The same reason promoting the tolerability of SSRIs in clinical practice might be the factor leading to their inferiority in the management of chronic pain conditions, as their selectivity to inhibit one monoamine system makes them less efficacious in treating chronic pain conditions as compared to TCAs (Barkin and Fawcett 2000). However and following the concept of individualized patient care, SSRIs could be beneficial in fibromyalgia patients presenting concurrent depressive symptoms and they remain to be the drugs of choice in patients who are not able to tolerate other antidepressants.

Trazodone, an old second generation antidepressant with significant sedative activity, is frequently used, in an off-label basis, in the treatment of insomnia in many countries (Mittur 2011). A small two-month double-blind controlled crossover study, only published in an abstract form, found that trazodone use in fibromyalgia was associated with improvements in sleep disturbances, but had not any significant effect on psychological profile and clinical symptoms (Branco 1996). Later, two uncontrolled studies have been published. The first of them found that trazodone, in a flexible dose

up to 300 mg/daily, improved sleep quality, anxiety and depression but had no effect on pain (Morillas-Arques et al. 2010). The second one found that the addition of pregabalin to trazodone treatment potentiated the antidepressant efficacy and improved pain, with the combination of the two drugs being well tolerated (Calandre et al. 2011).

1.9.1.2. Anti-epileptics

To use term antiepileptics is probably misleading since only one class of antiepileptic drugs, those of calcium channel modulators, has been shown to be useful in the management of fibromyalgia. Pregabalin and gabapentin play a critical role in pain perception through the modulation of α2δ voltage-gated calcium channel that leads to the inhibition of the synaptic release of glutamate, substance P and other neurotransmitters mediating pain response (Tuchman et al. 2010). They are widely used in several chronic pain conditions both in Europe and in the United States. Pregabalin is FDA-approved for the treatment of diabetic peripheral neuropathy, postherpetic neuralgia, neuropathic pain due to spinal cord injury and fibromyalgia, and EMA-approved for peripheral and central neuropathic pain, whereas gabapentin is FDA-approved only for the treatment of postherpetic neuralgia and EMA-approved for the treatment of peripheral neuropathic pain. Pregabalin shows better pharmacokinetic and pharmacodynamic profiles (Bockbrader et al. 2010) making it preferable to gabapentin (studied in only one small 12 week randomized clinical trial) that could be considered in patients who are responsive but cannot tolerate pregabalin. Their use in fibromyalgia is supported by two recent meta-analyses (Hauser et al. 2009, Tzellos et al. 2010). Both drugs have been found to reduce pain and to improve sleep and health-related quality of life; however, they showed non-substantial effect on fatigue and anxiety and lacked effect on depression with adverse events that included dizziness, somnolence, dry mouth, weight gain and peripheral edema. The evidence for pregabalin efficacy is stronger than the one for gabapentin.

1.9.1.3. Analgesics

1.9.1.3.1. Non-steroidal antiinflammatory drugs (NSAIDs):

Although pain is the cardinal symptom of fibromyalgia, NSAIDs do not contribute significantly to its management, which is not surprising given the non-inflammatory nature of the disease. Although the evidence concerning their effectiveness in the treatment of fibromyalgia is limited, controlled trials have shown little or no efficacy either when administered alone or combined with other drugs such as amitriptyiline or benzodiazepines (Goldenberg et al. 1986, Yunus et al. 1989, Russell et al. 1991, Quijada-Carrera et al. 1996). However, they are still widely used, although they are not perceived among the most effective medications (Bennett et al. 2007).

1.9.1.3.2. Opioids

Clinical trials evaluating the efficacy of opioids are limited to a very small double-blind trial of intravenous morphine showing no efficacy and low tolerability. However, despite the fact that there is no convincing evidence of efficacy, opioids are frequently prescribed for the treatment of pain in fibromyalgia in many countries (Ngian et al. 2011). An uncontrolled study, published only in abstract form, did not find evidence of pain improvement during a four-year follow-up of fibromyalgia patients treated with opioids but found that, after two years of treatment, depression increased (Kemple 2003). The use of opioids in fibromyalgia is also problematic due to their side-effect profile; in addition to the risk of dependence, there is also of concern the constipation, considering that this is a very common symptom reported by patients with fibromyalgia whose comorbidity with irritable bowel syndrome is high; likewise, opioids sedation and mental clouding may worsen the cognitive dysfunction experienced by many patients (Ngian et al. 2011).

1.9.1.3.3. Other analgesics

Tramadol is a drug that acts both as agonist on μ opioid receptors and as inhibitor of serotonin and noradrenaline reuptake. It has been shown to be effective in the management of pain in fibromyalgia both as monotherapy (Russell et al. 2000) and combined with paracetamol (Bennett et al. 2003). The use tramadol or tramadol-

paracetamol combination for the treatment of pain should be better reserved for “as needed” medication, for periods of fibromyalgia flare-ups, rather than for chronic use.

Tapentadol, a centrally-acting analgesic, is structurally related to tramadol; it has higher affinity than tramadol for μ receptors and inhibits the reuptake of noradrenaline but not of serotonin (Raffa et al. 2012). It has shown to be effective in different types of chronic non-cancer pain with better tolerability than pure μ-opioid receptor agonists (Pergolizzi et al. 2012). Although it seems a promising drug for the treatment of pain in fibromyalgia, its potential role in this indication awaits further investigation.

1.9.1.4. Other drugs

1.9.1.4.1. Cyclobenzaprine

Cyclobenzaprine, licensed as a muscle relaxant, is an old drug structurally similar to TCAs. In a meta-analysis including 5 randomized, placebo-controlled trials, cyclobenzaprine demonstrated effectiveness in improving sleep, and modestly improving pain in the early stages of treatment, but it didn’t improve fatigue or tender points (Tofferi et al. 2004). Favorable outcomes of very low dose cyclobenzaprine (1-4 mg) were achieved in an 8-week, double-blind, placebo-controlled trial, where it was associated with significantly improved pain, tenderness, depressive symptoms, and increased nights of restorative sleep (Moldofsky et al. 2011).

1.9.1.4.2. Sodium oxybate

Sodium oxybate, the sodium salt of gamma-hydroxybutyrate that gained FDA orphan drug status for the management of cataplexy and daytime sleepiness in patients with narcolepsy, has been shown to be effective for the treatment of fibromyalgia in four large randomized controlled clinical trials; however, it failed to gain the FDA approval for use in fibromyalgia due concerns of potential abuse (Staud 2011). In all of these studies, sodium oxybate improved outcomes of pain, fatigue, sleep disturbances, Fibromyalgia Impact Questionnaire scores and the patient global impression of change (Swick 2011).

1.9.1.4.3. Sedative-hypnotics

The suggested role of sedative hypnotics in the management of fibromyalgia arises from the major sleep disturbance that this group of patients experiences. Knowing that a positive association exists between poor sleep and pain and fatigue (Nicassio et al. 2002), improvement of sleep symptoms was expected to be associated with better outcomes on pain and other fibromyalgia symptomatology. However, some benzodiazepines, temazepam, alprazolam, and bromazepam, have been tried in the treatment of fibromyalgia with little or no result (Russell et al. 1991, Quijada-Carrera et al. 1996, Lawson 2008). Also, the two non-benzodiazepine GABA-A agonists zolpidem and zopiclone, improved sleep but neither pain nor mood disturbances (Drewes et al. 1991, Gronblad et al. 1993, Moldofsky et al. 1996). Thus, it seems that the role of these drugs in the management of fibromyalgia is very limited.

1.9.1.4.4. Dopamine agonists

The evidence for a possible role of the D2 dopaminergic agonists in the treatment of fibromyalgia is conflicting. A randomized controlled trial with pramipexole found that the drug improved pain, fatigue and global well-being (Holman and Myers 2005). However, two additional clinical trials evaluating ropinirole and extended-release ropinirole failed to find any treatment-related benefit (Wood and Holman 2009). One trial with extended-release pramipexole (NCT00689052) was early terminated (Boehringer 2008) and other with rotigotine (NCT00464737), has been completed but not published (Pharma 2008).

1.9.1.4.5. 5-HT3 antagonists

Several trials with intravenous or oral tropisetron and one with intravenous dolasetron in the treatment of fibromyalgia have been published showing a significant decrease of pain levels. The use of these drugs has been advocated mainly for patients suffering high levels of pain and not showing relevant psychological distress (Seidel and Muller 2011).

1.9.1.5. One drug or combination therapy?

As there is not a drug able to control all the symptoms of the disease, the possibility to administer more than one drug simultaneously seems logical and, in fact, and several studies have found that in the routine clinical practice, patients receive a mean of 2-3 concomitant drugs (Wolfe et al. 1997, White et al. 2002, Wolfe et al. 2010). However, and in contrast with the abundance of published monotherapy clinical trials, little information is available concerning the efficacy and tolerability of drug combinations. Thus, when considering polytherapy, therapeutic decisions must be based on data from monotherapy trials and a sound knowledge of the pharmacological profile of each drug in order to combine drugs that improve different symptoms of the disease while avoiding the overlapping of side effects (Calandre et al. 2012).

1.9.2. Non-pharmacologic Therapy

Several non-pharmacologic treatment options have been shown to be effective as part of the management of fibromyalgia, and they are currently regarded as fundamental components in the treatment plan of fibromyalgia patients.

1.9.2.1. Education

Education is viewed as a basic component of a well-designed therapeutic plan in fibromyalgia. This intervention is characterized by an informative role that aims toward increasing patients' awareness about the various aspects of the disease, such as the possible pathogenesis and its correlation with the different symptoms, the role of pharmacologic therapies in fibromyalgia and the extent of the benefit they provide, in addition to the possible lifestyle factors and activities that might alleviate or exacerbate fibromyalgia symptoms. However, clinical trials assessing the role of education in fibromyalgia have evaluated this intervention mostly as an add-on of other non-pharmacologic therapies except in four controlled clinical studies that evaluated the discrete role and benefit of education. Burckhardt et al. (1994) compared education alone and education plus physical training with a waiting-list control group and found a significant positive impact on quality of life and self-efficacy for both interventions as compared to the control group. Bosch Romero et al. (2002) evaluated the impact of the

education versus no intervention in two groups of patients with fibromyalgia; they found that the education group improved only in the perception of body pain. Rooks et al. (2007) compared education alone with two kinds of exercise programs and the combination of education and exercise; they found all of four groups improved but that the degree of improvement was highest in the combination group. Stuifbergen et al. (2010) compared education alone with education plus lifestyle changes and again it was found that both groups improved along the time but that the combined group improved more in physical activity and stress management.

Although the available evidence is limited, the results of these studies support a modest beneficial role of education that also seems to potentiate other non-pharmacologic interventions. Thus, education would be mostly indicated in multimodal therapeutic interventions.

1.9.2.2. Exercise

The role of exercise has been widely studied for its potential benefits in fibromyalgia. The types of exercise interventions that have been investigated in fibromyalgia include aerobic exercise (land-based and water-based), strength, and flexibility, and different combinations of these, with aerobic exercise being the most investigated intervention. Exercise interventions in fibromyalgia have been generally found to be associated with decreased pain intensity, reduced severity of fibromyalgia symptoms, and improved emotional and mental health (Busch et al. 2011). Combined exercise modalities and aerobic exercise are the interventions that have the strongest evidence of benefit in patients with fibromyalgia (Jones and Liptan 2009). A meta-analysis evaluating the effects of the different types of aerobic exercise (land-based and water-based) showed that both types of physical activities significantly improved pain, depressed mood, fatigue, quality of life and physical fitness but had non-significant influence on sleep disturbances(Hauser et al. 2010). A review aiming to evaluate which kind of physical exercise is best for fibromyalgia found that there were not clear differences in the efficacy of either land-based aerobic exercise, water-based aerobic exercise and muscle strengthening, although water-based exercise and strengthening seemed to be slightly more effective in reducing spontaneous pain and depressive mood (Cazzola et

al. 2010). Despite not being frequently reported, adverse effects associated with exercise therapy such as increase in symptoms (pain, stiffness and fatigue) and musculoskeletal problems should be also evaluated and considered (Busch et al. 2011). Accordingly, it is recommended to consider individualized plans of exercise therapies that are primarily determined by the patients' preference and accessibility; these plans should cover the specific physical needs of the patient and ensure adequate compliance in order to avoid attrition that is frequently reported.

1.9.2.3. Psychotherapy

The most studied psychotherapeutic approach in fibromyalgia is the cognitive behavioral therapy (CBT). CBT combines cognitive and behavioral therapies that are intended to assist patients in building a set of skills to encounter dysfunctional emotions, thoughts and behaviors (Cully 2008). Pain catastrophizing represents the characterization of pain as being awful, horrible and unbearable, a feature that suggests psychological vulnerability and constitutes a major contributor to the exaggeration of pain experience and depression (Gracely et al. 2004).Compared to patients with rheumatoid arthritis, fibromyalgia patients show a significantly more prominent catastrophizing profile, suggesting the need for cognitive therapy and coping skills (Hassett et al. 2000). In a meta-analysis including 23 studies that evaluated different psychological treatments in patients with fibromyalgia, psychotherapy effectively reduced sleep problems, depression, functional status, and catastrophizing with CBT having the greatest effect size compared to other psychological interventions (relaxation, education, behavioral treatments, mindfulness-based treatments, and eye movement desensitization and reprocessing) (Glombiewski et al. 2010). In another meta-analysis including 14 randomized clinical trials specifically investigating the efficacy of CBT in fibromyalgia, CBT was associated with significant decrease in depressive symptoms, self-efficacy pain (i.e. subjects' perceived ability to manage and cope with pain and its emotional and behavioral consequences) and the number of physician visits at follow-up; however, non-significant effects on pain, sleep, fatigue and health-related quality of life were obtained (Bernardy et al. 2010).Therefore, it can be

concluded that psychotherapeutic intervention remains an essential component in the treatment of fibromyalgia given the psychological vulnerability of fibromyalgia patients.

1.9.3. Complementary and Alternative Medicine

As there is no cure for fibromyalgia and as the available therapies offer only partial efficacy, patients frequently turn to complementary and alternative medicine (CAM) looking for additional relief (Pioro-Boisset et al. 1996, Lind et al. 2007). CAM is a broad term which encompasses a huge variety of therapies that are not generally considered to be part of the conventional medicine and that patients can use together with it (hence the expression complementary) or in its place (hence the expression alternative).The U.S. National Institutes of Health broadly classifies CAM in the following four categories (NCCIH 2008):

- natural products: such as herbal medicines, vitamins, minerals, dietary supplements and probiotics.

- mind and body medicine: including acupuncture, relaxation techniques, qi gong, tai-chi or hypnotherapy among the most known.

- manipulative and body-based practices: including spinal manipulation and massage therapy among the most frequently used.

- other CAM practices: that include movement therapies, traditional healers practices, energy fields, and whole medical systems.

Many CAM therapies have never been adequately investigated for efficacy and among those that have been studied, there were usually few trials of small sample size. A meta-analysis of CAM in the treatment of fibromyalgia found that only balneotherapy had effect sizes indicating that they reduced pain in fibromyalgia (Terhorst et al. 2011). Massage, acupuncture and nutritional supplements showed no clear evidence of efficacy. Finally, manipulative, vibration, magnetic, homeopathy, movement therapy and energy medicine has 3 or less trials and could not be analyzed. Thus, it seems clear

that additional studies are needed to evaluate the values of the different CAM therapies in the management of fibromyalgia.

1.9.4. Multi-component Treatment

The complexity of fibromyalgia and its overlapping pathophysiological mechanisms makes difficult to control the broad range of the disease symptoms. All of the pharmacologic and non-pharmacologic options previously mentioned are associated with a limited extent of improvement in fibromyalgia symptomatology, and none of them provides sufficient benefit when prescribed alone. Therefore, it is widely accepted among healthcare professionals that a patient-specific multi-component therapeutic approach including both non-pharmacologic and pharmacologic therapies should be employed to attain optimal clinical outcomes. Given the distinct mode of activity of each type of intervention, it makes sense to combine them for obtaining a maximal benefit of symptomatic improvement. In a meta-analysis including 9 RCTs with 1,119 subjects, it was shown that a multi-component therapy with at least 2 non-pharmacologic therapies (at least 1 educational or other psychological therapy and at least 1 exercise therapy) was associated with strong evidence for reducing pain, fatigue, depressive symptoms, physical fitness and limitations to health-related quality of life (Hauser et al. 2009). In a recent randomized clinical trial assessing the efficacy of multidisciplinary treatment (conventional pharmacologic treatment, CBT, and physical therapy) versus a control group receiving conventional pharmacologic treatment only in women with low educational level, it was found that improvements in functionality, sleep disturbances, pain intensity, catastrophizing and psychological distress were significantly superior in the multidisciplinary treatment group and that improvements of sleep disturbances, catastrophizing and psychological distress were maintained at 12-month follow-up (Castel et al. 2013). The strength of evidence supporting the adaptation of multidisciplinary approach in fibromyalgia is a moderate to strong evidence; however it is not yet known which combinations best provide the optimal benefit in fibromyalgia (Sarzi-Puttini et al. 2008). Various combination approaches are possible, and the selection of these options relies on the specific patient’s needs on one hand and on the patient’s accessibility to the suggested treatment options on the other hand. At least, the

combination of pharmacologic therapy and exercise should be mandatory; patients' education and/or CBT should be added whenever possible.

1.10. Fibromyalgia and the gastrointestinal dimension

Gastrointestinal manifestations, frequently reported by patients with fibromyalgia, were first described by Yunus et al. in 1981 and were referred to as IBS-like symptoms (Yunus et al. 1981). The common comorbidity of fibromyalgia and irritable bowel syndrome, which has been shown to range between 32% and 81% depending on the applied diagnostic criteria (Veale et al. 1991, Sperber et al. 1999, Kurland et al. 2006), has been suggested as a possible explanatory mechanism underlying the occurrence of these symptoms in fibromyalgia. However, a considerable proportion of patients with fibromyalgia present non-specific gastrointestinal symptoms such as abdominal pain, dyspepsia, belching, bloating, sour taste, alternation of diarrhea-constipation, abdominal gas and nausea (Triadafilopoulos et al. 1991, Pamuk et al. 2009), which are not sufficient to establish a diagnosis of irritable bowel syndrome. This indicates the possible presence of other underlying factors, besides the irritable bowel syndrome comorbidity, contributing to the appearance of these manifestations.

1.10.1. Gastrointestinal manifestations in fibromyalgia

In the first controlled study conducted in fibromyalgia by Yunus et al. (1981), the authors reported a significantly elevated incidence of gastrointestinal symptoms among patients with fibromyalgia as compared to control subjects (34% versus 8% respectively; $P< 0.01$). This pioneer study was followed by several investigations that evaluated the occurrence of gastrointestinal manifestations in fibromyalgia. The majority of these studies focused on the comorbidity between irritable bowel syndrome and fibromyalgia.

Initial reports concerning this comorbidity date back to the early 1980s starting with Campbell et al. (1983) who reported the occurrence of symptoms compatible with irritable bowel disease in 50% of patients with fibrositis compared to 5% of controls followed by Bengtsson et al. (1986) who in turn reported their occurrence in 44% of patients with primary fibromyalgia.

The frequent occurrence of gastrointestinal symptoms among patients with fibromyalgia led to the initial proposal of assuming irritable bowel symptoms as a minor criterion for the diagnosis of fibromyalgia syndrome (Goldenberg 1987). This suggestion could be justified by the elevated prevalence of comorbid irritable bowel syndrome in fibromyalgia. The first study to evaluate the frequency of this prevalence was conducted by Romano who reported the diagnosis of irritable bowel syndrome in 49% of patients with primary fibromyalgia, 19% of patients with secondary fibromyalgia, and 9% of arthritic controls (Romano 1988).

Another study that aimed to estimate the prevalence of irritable bowel syndrome in patients with primary fibromyalgia and vice versa adopted stricter criteria for the diagnosis of irritable bowel syndrome, which was based on the history of irritable bowel syndrome symptoms, physical examination and sigmoidoscopy if indicated (Veale et al. 1991). Five groups were recruited, each consisting of 20 patients: primary fibromyalgia, irritable bowel syndrome, inflammatory arthritis, inflammatory bowel disease and a control group of healthy subjects (Veale et al. 1991). Seventy percent of patients with fibromyalgia were diagnosed with irritable bowel syndrome and 65% of patients with irritable bowel syndrome were diagnosed with fibromyalgia. On the other hand, the remaining three groups, combined, had a total of 12% and 10% prevalence of fibromyalgia and irritable bowel syndrome respectively. The authors concluded a possible presence of common pathogenetic mechanisms for both fibromyalgia and irritable bowel syndrome. However, a major limitation to this study was the small size of the evaluated samples.

In another study that examined the possible association between fibromyalgia and other medical and psychiatric disorders in a sample of 33 women with fibromyalgia, current and life-time diagnoses of irritable bowel syndrome were seen in 39% and 52% of patients with fibromyalgia respectively (Hudson et al. 1992). A comparable rate of irritable bowel syndrome diagnosis was also reported by Nishikai et al. (1992) as it was established in 50% out of the 50 participating subjects with fibromyalgia. These findings suggested the existence of an impairment at the intestinal level among patients with fibromyalgia that Sivri et al. (1996) attributed to a more distressing bowel pattern and a

different threshold of sensitivity to bowel stimuli. Of the 75 patients with fibromyalgia evaluated by these authors, 41.8% experienced altered bowel function fulfilling criteria consistent with irritable bowel syndrome as compared to 16% of the 50 control subjects ($P<0.05$). Patients with fibromyalgia reported specific intestinal symptoms that included abdominal pain more than six times a year (38.2%), abdominal distention (45.5%), sense of incomplete evacuation (30.9%), mucus in the stool (9.1%), constipation (30.9%) and diarrhea (7.3%).

Two parallel studies were later conducted and aimed toward studying the prevalence of fibromyalgia among irritable bowel syndrome patients compared to matched controls (irritable bowel syndrome study) and the prevalence of irritable bowel syndrome among patients with fibromyalgia (fibromyalgia syndrome study) taking into consideration the influence of coexistent irritable bowel syndrome and fibromyalgia on health-related quality of life (Sperber et al. 1999). Compared to previous studies, relatively modest prevalence rates were obtained as 32% of patients with fibromyalgia had a diagnosis of irritable bowel syndrome and 31.6% of patients with irritable bowel syndrome had fibromyalgia (Sperber et al. 1999). With respect to the impact on the health-related quality of life in the irritable bowel syndrome study, patients with concomitant fibromyalgia and irritable bowel syndrome had significantly worse outcomes in terms of global well-being, sleep disturbances, physician visits, pain, anxiety and global severity index. Similarly, patients with fibromyalgia and irritable bowel syndrome in the fibromyalgia study had significantly worse physical functioning scores and overall quality of life scores (Sperber et al. 1999).

The first study to evaluate the possible association of fibromyalgia with the type and severity of irritable bowel syndrome was done by Lubrano et al. (2001). Irritable bowel syndrome was classified into four different patterns based on the predominant bowel symptom: diarrhea predominant, constipation predominant, alternate constipation and diarrhea, and abdominal pain predominant; severity classification of irritable bowel syndrome, based on a functional severity index, comprised three different grades: mild, moderate and severe. Twenty percent of the 130 subjects who fulfilled the Rome I criteria for the diagnosis of irritable bowel syndrome were diagnosed with fibromyalgia

based on ACR 1990 criteria. No association was found between the presence of fibromyalgia and the type of irritable bowel syndrome, whereas a significant association was noticed between the presence of fibromyalgia and irritable bowel syndrome severity, with a significant positive correlation being found between the number of tender points and the severity of irritable bowel syndrome (Lubrano et al. 2001).

Following the evolvement of Rome II criteria as a result of the 1999 revision made to the initial Rome criteria issued for the diagnosis of functional gastrointestinal disorders, Kurland et al. (2006) were the first to study the prevalence of irritable bowel syndrome in fibromyalgia taking into consideration the differences between Rome I and Rome II criteria. Out of the 105 patients with fibromyalgia, irritable bowel syndrome was diagnosed in 63% of subjects using Rome I and 81% of subjects using Rome II criteria compared to 15% and 24% of rheumatologic control subjects respectively (n=62) ($P<0.001$) (Kurland et al. 2006).

In a retrospective study tracking data of 97,593 patients with irritable bowel syndrome and 27,402 non-irritable bowel syndrome controls, the authors evaluated the prevalence of functional somatic syndromes (including fibromyalgia) among patients with irritable bowel syndrome compared to non-irritable bowel syndrome subjects; the OR of fibromyalgia was 1.8 (95% CI 1.7 – 1.9) in the irritable bowel syndrome subjects as compared to control subjects (95% CI 1.7 – 1.9) (Cole et al. 2006). In another attempt to assess the comorbid existence of gastrointestinal disorders, psychiatric disorders and non-gastrointestinal somatic disorders in irritable bowel syndrome, a systematic review reported a 32.5% prevalence of fibromyalgia in patients with irritable bowel syndrome (estimated range: 28%-65%) and 48% prevalence of irritable bowel syndrome in patients with fibromyalgia (estimated range: 32%-77%) (Whitehead et al. 2002).

Besides the studies associating the appearance of such gastrointestinal manifestations to the potential comorbidity with irritable bowel syndrome, several other studies have investigated the frequency of such symptoms in a non-specific approach. Among these studies is the first extensive evaluation of the prevalence of these symptoms in fibromyalgia conducted by Triadafilopoulos et al. (1991). A validated self-administered

questionnaire to evaluate the prevalence of symptoms of bowel dysnfunction and irritable bowel syndrome was completed by 123 patients with fibromyalgia, 54 patients with degenerative joint disease, and 46 controls (Triadafilopoulos et al. 1991). Altered bowel function was seen in 73% of patients with fibromyalgia compared to 37% of patients with degenerative joint disease and 0% of control subjects ($P< 0.001$). Alternating diarrhea-constipation was in turn seen in 63% of patients with fibromyalgia compared to 22% of patients with degenerative joint disorder and 0% of controls ($P< 0.001$). Other gastrointestinal complaints reported by patients with fibromyalgia included abdominal gas (59%), nausea (21%), diarrhea (9%), and constipation (12%) (Triadafilopoulos et al. 1991). During periods of disease exacerbation, bowel complaints were worse among 50% of patients with fibromyalgia compared to 28% of patients with degenerative joint disorders ($P<0.05$) and another interesting observation was the use of laxatives among patients with fibromyalgia (19% versus 0% in the other two groups) (Triadafilopoulos et al. 1991).

In spite of the extensively studied correlation between fibromyalgia and irritable bowel syndrome, little has been published regarding the correlation between fibromyalgia and other inflammatory and functional bowel disorders. While a contradictory evidence exists about the association of fibromyalgia with inflammatory bowel disease (Wallace and Hallegua 2004), the majority of patients with fibromyalgia present with at least one functional gastrointestinal disorder (Almansa et al. 2009). Almansa et al. (2009) found a 98% prevalence of at least one functional gastrointestinal disorder (esophageal, gastroduodenal, bowel or anorectal) among the 100 patients with fibromyalgia compared to 39% of the 100 matched controls. Among the different functional gastrointestinal disorders, the strongest association was seen with irritable bowel syndrome (prevalence 39%, 95%CI: 29.4–48.6) followed by functional bloating (34%; 95%CI: 24.7-43.3). At the level of the potential role played by the psychologic features in the relationship between these conditions, patients with fibromyalgia had significantly higher Symptom Checklist-90 Revised (SCL-90R) scores compared to controls except for hostility, phobia, paranoia, pyschoticism, and positive symptom total; these scores were especially higher among those with comorbid fecal incontinence (Almansa et al. 2009).

In the most recent study evaluating the frequency and severity of gastrointestinal symptoms in fibromyalgia taking into consideration their influence on the quality of life, significantly higher frequencies of belching, reflux, bloating, sour taste, and vomiting were seen among patients with fibromyalgia compared to patients with rheumatoid arthritis and controls (P< 0.01) (Pamuk et al. 2009). Disturbances in the dyspepsia-related quality of life were significantly higher among patients with fibromyalgia compared to the other two groups (P<0.01). Although the frequency of constipation was significantly higher in the rheumatoid arthritis group (49%) as compared to fibromyalgia (29.6%) and controls (23.3%) (P<0.01), higher elevation in the constipation-related quality of life disturbances was seen among patients with fibromyalgia (Pamuk et al. 2009).

1.10.2. Fibromyalgia and irritable bowel syndrome: Resemblance and divergence

The resemblance between the two syndromes is not limited to the overlapping manifestations that have been already highlighted. Both fibromyalgia (Queiroz 2013) and IBS (Adeyemo et al. 2010) are linked to an increased prevalence among females. Akkuş et al. (Akkus et al. 2004) attributed this elevated prevalence of IBS among females to coexisting fibromyalgia. However, a meta-analysis conducted by Lovell et al. (Lovell and Ford 2012) showed only a modest predominance of IBS among females (OR: 1.67, 95% CI: 1.53-1.82). Similarly, in fibromyalgia, the female predominance can be lower and less striking to what has been previously reported; a recently published study found a 2.4% prevalence of fibromyalgia in females compared to 1.8% in males (P= 0.372) (Wolfe et al. 2013).

In his review article, Chang (1998) details several grounds to support the claim of a common etiology for fibromyalgia and irritable bowel syndrome. These include the common features shared by the two syndromes such as the exacerbation of symptoms with stressful life events, the complain of disturbed sleep and fatigue by the majority of patients, the efficacious treatment of symptoms through psychotherapy and behavioral therapies in addition to the improvement of the irritable bowel syndrome symptoms with low dose tricyclic antidepressants.

On the other hand, differences exist between the two conditions, as the different response to somatic and visceral stimuli reported by Chang; while the response to mechanical stimuli is manifested as somatic hyperalgesia in fibromyalgia, patients with irritable syndrome without coexistent fibromyalgia exhibit somatic hypoalgesia (Chang 1998). Moreover, differing perceptual alterations between patients with irritable bowel syndrome and those with fibromyalgia have been documented in visceral distention studies (Chang 1998). This perceptual difference was further confirmed in a study conducted by Caldarella et al. (2006), where rectal distensions generated hypersensitivity in patients with irritable bowel syndrome and those with coexistent irritable bowel syndrome and fibromyalgia, however patients presenting with only fibromyalgia well-tolerated all distensions without discomfort. This finding suggests the presence of multiple mechanisms that modulate perceptual somatic and visceral responses (Caldarella et al. 2006).

Given the previously detailed claims of the possible overlapping pathophysiologic mechanisms between fibromyalgia and irritable bowel syndrome, Reitblat et al. (2009) conducted a 1-month prospective study to evaluate the effect of the tegaserod (a 5HT4 partial agonist used for the management of irritable bowel syndrome with constipation) in 14 females suffering from fibromyalgia and constipation dominant irritable bowel syndrome (Reitblat et al. 2009). The irritable bowel syndrome status, the total fibromyalgia impact questionnaire score, the number of tender points and pain in tender points decreased significantly after the treatment ($P<0.01$). However the major limitation of this study was the poor generalizability caused by the uncontrolled study design, small sample size, and short duration of study (Reitblat et al. 2009).

1.11. Dietary interventions in Fibromyalgia

A considerable percentage of patients with fibromyalgia believe that dietary interventions have a great influence on the disease symptoms and perceive symptomatic aggravation as being secondary to the intake of specific foods (Haugen et al. 1991). Accordingly, a general tendency exists among these patients toward adopting dietary interventions in order to attain better symptomatic control. Modifications of the dietary habits have been shown to be adopted by up to 30% of patients with

fibromyalgia (Arranz et al. 2012); these authors also found that 7% of the patients reported to have been diagnosed of food allergy or intolerance. Conversely, symptoms suggestive of fibromyalgia were found in the 71% of a sample of 84 patients experiencing perceived food hypersensitivity (mainly to bread, milk and fruits). In a recent study investigating food allergy in fibromyalgia, 49% of cases reported the presence of food allergy and 66% of them reported the appearance of symptoms with milk, wheat and orange (Puccio et al. 2013).

The existing notion of the beneficial role of dietary interventions among patients with fibromyalgia has promoted the common adoption of dietary modifications based on an individual initiative. In this regard, several attempts were undertaken to investigate the benefit of the commonly selected dietary interventions such as vegetarian diet, Mediterranean diet, vegan diet, elimination diet, hypocaloric diet and gluten-free diet with variable outcomes being reported.

1.11.1. Vegetarian diet/ Mediterranean diet

The effects of vegetarian diet in fibromyalgia have been explored in one open-label randomized clinical trial (Azad et al. 2000) and in another observational study (Donaldson et al. 2001). In the first study, the authors mainly aimed to assess whether the reduction of the intake of protein rich in neutral amino acid (reported to lower tryptophan levels in brain), through the exclusion of animal protein, would improve fibromyalgia symptomatology. Patients with fibromyalgia were randomly allocated to a 6-week vegetarian diet intervention (n= 37) or pharmacologic amitriptyline therapy (n= 41). While significant improvement were encountered in the amitriptyline group at the level of pain, fatigue, insomnia and non-restorative sleep, insignificant changes were seen in the dietary group except for a slightly significant drop in the pain scores; however, changes in the pain score were much smaller than those observed in patients in the amitriptyline group (Azad et al. 2000). Conversely, in the observational study conducted by Donaldson et al. (2001), the adoption of a vegetarian diet over a period of 6 months was associated to significant drop in the FIQ score (46% drop) in addition to significant improvements in the SF-36 scores, quality of life and physical performance. Considering the positive outcomes of this second study, Bennett (2002) claimed the

need of controlled clinical trials to confirm the benefit of this dietary intervention as compared to other dietary interventions that could serve as a control.

The evaluation of the effects of vegetarian Mediterranean diet compared to extended fasting, in a non-randomized study, on the intestinal flora, immunoglobulin A secretion and clinical outcomes among patients with fibromyalgia or rheumatoid arthritis demonstrated the lack of any significant improvement in none of these outcomes. However, the authors recommended further testing of these diets in randomized trials (Michalsen et al. 2005).

1.11.2. Vegan diet

The effects of the vegan diet on the symptoms of fibromyalgia have been evaluated in a non-randomized controlled clinical study (Kaartinen et al. 2000). A strict, low-salt, uncooked vegan diet rich in lactobacteria (n=18) was compared against omnivorous diet (n= 15); the adoption of this vegan diet by patients with fibromyalgia over a period of 3 months led to significant improvements in the pain VAS, joint stiffness, sleep quality and general health status. Interestingly, these positive outcomes disappeared gradually after shifting back to the omnivorous diet.

1.11.3. Elimination diets

Several dietary elements have been thought of as possible stimulants of fibromyalgia symptoms. Accordingly, various attempts have been made to evaluate the benefit of elimination diets in fibromyalgia, excluding various dietary elements such as monosodium glutamate (MSG), aspartame, refined and added simple sugars, caffeine, seafood, gluten, eggs and dairy products.

Using an individualized elimination diet, whose nature is dependent on the reactivity results displayed by lymphocyte response array, combined with a variety of supplements (antioxidants, buffering minerals, metabolic intermediates and necessary cofactors), (Deuster and Jaffe 1998) reported 50% decrease in the intensity of pain, 70% less depression, 30% less stiffness and 50% more energy, whereas, subjects in the control group didn’t demonstrate any improvement.

Smith et al. (2001) postulated that MSG and aspartame, which could act as excitatory neurotransmitters, can contribute to neurotoxicity when used in excess and consequently lead to the appearance of fibromyalgia symptoms. The exclusion of these "excitotoxins" from the diet of four patients diagnosed with fibromyalgia led to the complete or nearly complete resolution of their symptoms (Smith et al. 2001). In a more recent study conducted by Holton et al. (2012) that aimed to assess the effect of MSG challenge as compared to placebo among patients with fibromyalgia and IBS (n= 57) who were placed on a 4-week diet excluding excitotoxins of MSG and aspartame. Of the 37 patients who completed the study, 31 reported significant clinical improvement (> 30%). These patients underwent a 2 week placebo-controlled crossover challenge with MSG for 3 consecutive days each week. The challenge of MSG resulted in worsening of fibromyalgia severity and QOL as compared to placebo, suggesting a possible role for dietary glutamate in fibromyalgia (Holton et al. 2012). On the other hand, Geenen et al. (2004) assert that dietary reduction of glutamate is not associated to pain relief in patients with fibromyalgia due to the independent CNS glutamate levels relative to serum concentration fluctuation following the ingestion of high concentrations of glutamate.

In a non-randomized study that included eight women with fibromyalgia, the adoption of a 4-week hypoallergenic diet and modified elimination diet (excluding seafood, refined and added sugar, artificial colorings, caffeinated beverages, gluten-containing grains, eggs, dairy products and allergenic foods) in addition to phytonutrient supplementation, a significant drop in FIQ and pain scores were reported (Lamb et al. 2011).

1.11.4. Hypocaloric diet

The correlations between fibromyalgia symptomatology and distorted quality of life with elevated body mass index have been demonstrated in several clinical trials (Yunus et al. 2002, Patucchi et al. 2003, Neumann et al. 2008, Okifuji et al. 2010, Arranz et al. 2012, Kim et al. 2012, Aparicio et al. 2014, Cordero et al. 2014). Additionally, in the study conducted by Neumann et al. (2008), obese patients with fibromyalgia displayed higher pain sensitivity and lower levels of quality of life. Subsequently, two prospective studies were performed to assess the effect of weight reduction (through hypocaloric

diet) in fibromyalgia (Shapiro et al. 2005, Senna et al. 2012). In the pilot study conducted by Shapiro et al. (2005), 42 patients were assigned to receive a 20-week behavioral weight loss treatment and a balanced deficit diet consisting of self-selected foods to remain within 1200–1500cal/day. The outcomes of this study indicated that although weight was correlated only to pain interference at baseline, the hypocaloric diet was effective in reducing pain, pain interference, and body dissatisfaction, as well as in improving QOL (Shapiro et al. 2005).

In the randomized clinical trial conducted by Senna et al. (2012), obese patients with fibromyalgia were randomly assigned to 6-month dietary weight loss group (n= 41) or no weight loss group (n= 42). As a result, patients in the weight reduction group had significant improvement in the overall FIQ score as compared to the control group. The FIQ subscales which displayed more significant improvement in the weight loss group included physical impairment, pain, fatigue, and depression. Moreover, significant improvements in depression, sleep quality and tender point count were seen in the weight loss group.

1.12. Hypothesis and Objectives

1.12.1. Rationale and Hypothesis

In clinical practice, a considerable overlap in the symptomatologic spectrum of fibromyalgia and gluten-related disorders (celiac disease or non-celiac gluten-sensitivity) can be noticed. Celiac disease and non-celiac gluten sensitivity (NCGS) share a wide array of gastrointestinal and extraintestinal manifestations such as diarrhea, abdominal pain, bloating, tiredness, fatigue, foggy mind, bone pain, headache, anemia, depression and anxiety (Nelsen 2002, Sapone et al. 2012, Pulido et al. 2013). In a recent cross-sectional study comparing the clinical manifestations among patients with fibromyalgia with those experienced by adult celiac patients and subjects with gluten sensitivity, a remarkable similarity of gastrointestinal and extraintestinal manifestations was found, whereby the frequency of presentation of every celiac-type symptom, excepting anemia, was significantly higher among patients with fibromyalgia as compared to controls ($p < 0.0001$) (Garcia-Leiva et al. 2015).

This symptomatologic overlap suggests a possible role of gluten sensitivity in at least a subgroup of patients with fibromyalgia who present gastrointestinal manifestations. This postulation can be supported by the increased susceptibility to multiple chemical sensitivity disorders (Slotkoff et al. 1997) and the frequent hypersensitivity to food components (Berstad et al. 2012, Puccio et al. 2013) among patients with fibromyalgia. NCGS has been linked to an underlying role in several disorders such as IBS and neuropsychiatric disorders (Catassi et al. 2013, Genuis and Lobo 2014) which indicates the possible presence of a broad range of disorders that could be affected by such sensitivity.

To date, scarce data exist concerning the potential outcomes linked to eliminating gluten from the diet of patients with fibromyalgia. In this regard, three studies have been published providing limited evidence regarding the exact role of gluten-free diet (GFD) in fibromyalgia. In a recent study conducted by Rodrigo et al. (2013), seven (6.7%) celiac cases were diagnosed among 104 patients with concomitant IBS and fibromyalgia. The adoption of a 12-month gluten-free diet by these seven patients led to significant clinical improvement of fibromyalgia symptoms, gastrointestinal manifestations and health-related quality of life seen (Rodrigo et al. 2013), emphasizing the close relationship existing between these conditions.

In another study conducted by Rodrigo et al. (2014), patients with fibromyalgia and IBS, who were placed on a 1-year GFD, demonstrated clinically significant improvement in their symptoms only if they were diagnosed with lymphocytic enteritis (LE), whereas those presenting normal intestinal mucosa didn’t show any benefit from the adoption of this diet. In the third study, preliminary results of the case-series report in a group of selected patients with fibromyalgia suggested that an improvement after gluten elimination from the diet can be seen (Isasi et al. 2014).

To date, the role of GFD in fibromyalgia has not been investigated in any randomized-controlled trial. The adoption of dietary interventions in the setting of a randomized double-blind clinical trial design is a challenging measure due to the nature of such intervention on one hand and the difficulty in selecting a placebo comparator

intervention on the other hand. While the nature of these interventions entails the implementation of an open-label design making unfeasible to implement blinding techniques, the second challenge represented in the appropriate selection of a control group could be solved by implementing an active-control comparator group using another potentially effective dietary therapy.

Given the suspected role for gluten sensitivity in eliciting the gastrointestinal discomfort experienced by patients with fibromyalgia and the previously reported benefits of weight loss in resolving fibromyalgia symptomatology, we aimed toward conducting a randomized and controlled pilot clinical trial that evaluates the effects of a gluten-free diet with respect to a hypocaloric diet among patients with fibromyalgia who present overlapping symptoms of gluten sensitivity.

1.12.2. Objectives

This pilot randomized-controlled clinical trial had as primary objective to investigate whether the adoption of GFD as compared to a hypocaloric diet, in patients with fibromyalgia who present overlapping manifestations of gluten sensitivity, is associated with a greater improvement in the number of experienced gluten sensitivity symptoms (gastrointestinal and extraintestinal). Secondary objectives include the evaluation of the effect of these dietary interventions on fibromyalgia symptoms (including the impact and the severity of the disease), sleep problems, pain intensity, depression, anxiety; and health-related quality of life.

2. Patients, Materials and Methods

2.1 Patients

The target population of our study were patients with fibromyalgia diagnosed according to the 2010 American College of Rheumatology (ACR) criteria (Wolfe et al. 2010). Participants were referred to our investigation group from patients' associations or by their physicians (primary care physicians, rheumatologists and pain clinics).

Inclusion criteria:

- Adult patients diagnosed with fibromyalgia according to the 1990 (Wolfe et al. 1990) or 2010 (Wolfe et al. 2010) American College of Rheumatology diagnostic criteria.
- Patients showing a minimum of 5 gluten sensitivity symptoms (intestinal and/or extraintestinal) following the completion of the screening list of symptoms; the list of symptoms and signs, presented in Table 3, was assembled given the elevated frequency of occurrence of such symptomatology in fibromyalgia reported in a previous study (Garcia-Leiva et al. 2015).
- Negative transglutaminase antibodies serological testing confirming the absence of celiac disease.
- Signed informed consent to participate in the clinical study (Annex I).

Exclusion criteria:

- Patients suffering from any disease that could prevent them from following any of the anticipated diet therapies.
- Current or previous history of substance abuse.
- Pregnant or lactating women.

2.2 Ethical aspects

Eligible patients were fully informed about potential risks and benefits of each of the dietary interventions. A detailed explanation of the all aspects of the study including the study rationale and objectives were undertaken by the study investigators. Participants were informed that the participation in the study was entirely voluntary and that they

may refuse to participate or withdraw from the study at any time. Only participants who were able and willing to provide a written informed consent were eligible to participate in the current clinical study. The study was approved by the Medical Ethics Committee at the University of Granada. The trial was registered in the online registry of the National Institute of Health (NIH) "www.ClinicalTrials.gov" with the registration number "NCT01881360".

2.3 Study Design

This pilot study was a single-center, randomized, open-label, parallel-group, 24-week clinical trial. Eligible patients were randomized to receive a gluten-free or a hypocaloric diet added to their current therapeutic regimen. All subjects underwent a baseline assessment during which an accurate evaluation of patients' eligibility to participate in the study, that included ruling out the presence of celiac disease by means of obtaining the antigliadin antibodies, was performed.

After two weeks, eligible patients were randomly assigned to receive one of the diet therapies, and a thorough explanation of the corresponding diet was carried out to reinforce the adequate adherence to the diet.

Patients were followed up over a total duration of 24 weeks. During the first 12 weeks, a follow-up by phone was performed two weeks following randomization and an evaluation visit was scheduled every 4 weeks. Patients were instructed to maintain their current therapeutic regimen without any modification. During the second 12 weeks of the study, an evaluation visit was scheduled every 6 weeks. Dosage adjustments of pharmacologic treatment, if necessary, could be made by the investigator on week 12 of the study and later maintained constant until the end of the study. The flow chart in Figure 2 illustrates the participants' flow throughout the study.

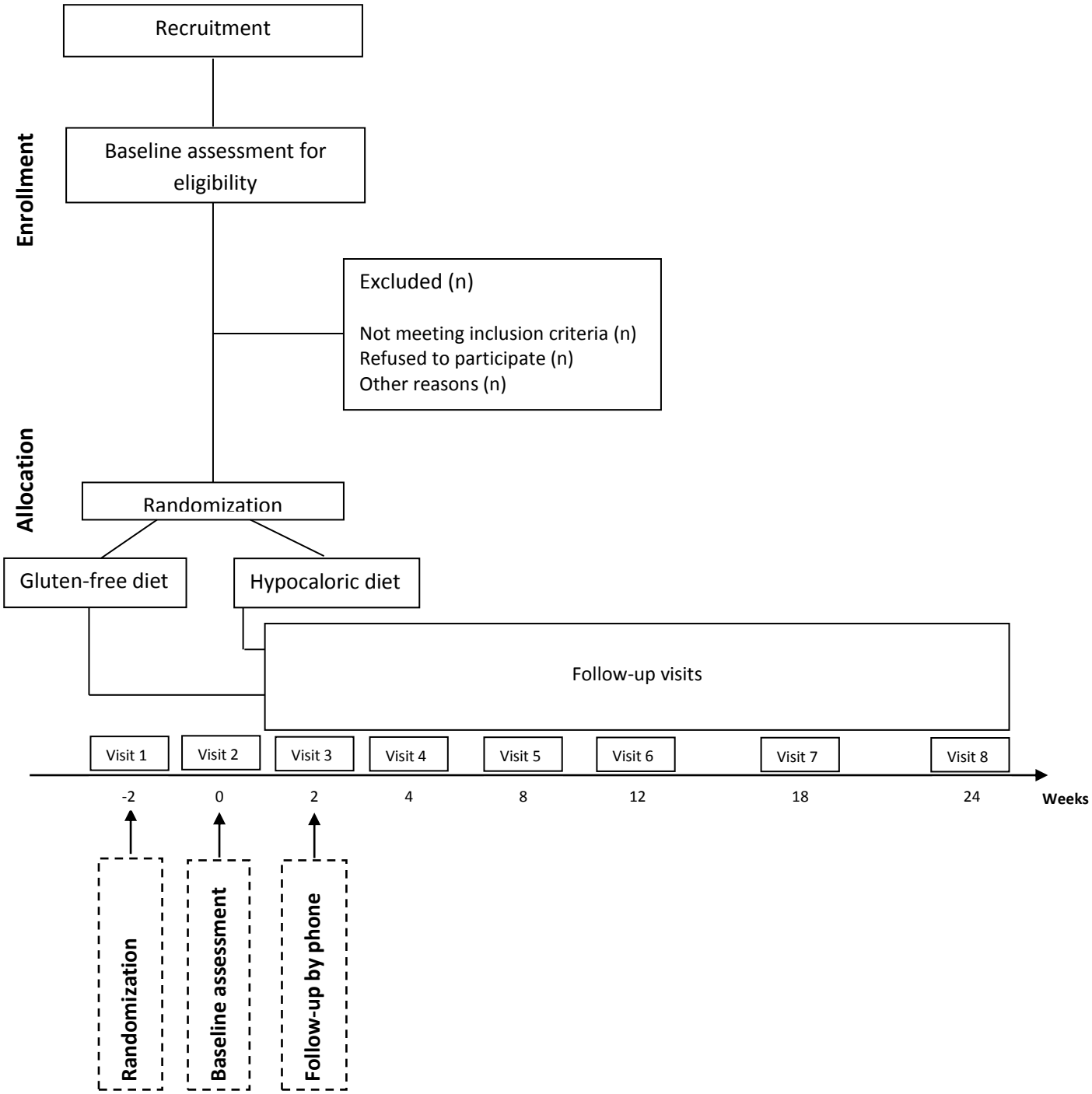


Figure 2. Flow chart of the study

The detailed chronogram of the study (Annex II) was as follows:

2.3.1 Baseline visit (Week -2)

During this visit, the patients were informed about the rationale behind conducting this study in addition to the objectives, nature of interventions, number of follow-up visits and the possible risks and discomfort. Subjects who showed interest to participate were asked to sign an informed consent form.

A thorough collection of the patients' demographic and clinical data, medical history, and current pharmacologic therapies was carried out. Accordingly, the patients' eligibility to participate in the clinical study was confirmed and that the patients fulfilled all the inclusion and none of the exclusion criteria. For this purpose, the investigators also ensured ordering a complete blood count, biochemistry and serology testing for each patient.

During the initial visit, the anthropometric measurements were collected and the patients were asked to complete the following questionnaires:

- List of gluten-sensitivity symptoms
- Revised fibromyalgia impact questionnaire (FIQR)
- Pittsburgh sleep quality index (PSQI)
- Brief pain inventory (BPI)
- Beck depression inventory-II (BDI-II)
- State-trait anxiety inventory (STAI)
- Short-form health survey (SF-12)
- Patient global impression scale of severity (PGI-S)

2.3.2 Allocation to the dietary therapies (Week 0)

After successfully completing the initial visit and confirming participation eligibility, the patients were randomly assigned to one of the dietary regimens (GFD or HCD) during

the second visit on week 0. A dietary orientation session was scheduled with each participant following the random allocation to one of the two interventions.

2.3.3 Follow-up by phone (Week 2)

Two weeks after the random allocation of participants to the corresponding dietary regimens, investigators contacted the patients by phone to check their adherence to the diet. Patients were asked about any doubts arising with the adherence to the newly assigned dietary therapies. The required clarifications were realized by the study investigators.

Patients were also asked about the occurrence of any possible adverse event.

2.3.4 Weeks 4 and 8 visits

Anthropometric measurements were performed and the patients were asked to complete the following questionnaires:

- List of gluten-sensitivity symptoms
- FIQR
- PSQI
- BPI
- BDI-II
- STAI
- SF-12
- PGI-S
- PGI-I

Patients were also asked about any doubts related to their diet and about the occurrence of any adverse event.

2.3.5 Week 12 visit

During this visit, slight modifications of the pharmacologic treatments, if needed, were made by the study investigators.

Complete blood count and biochemistry testing were ordered.
Anthropometric measurements were performed and the patients were asked to complete the following questionnaires:

- List of gluten-sensitivity symptoms
- FIQR
- PSQI
- BPI
- BDI-II
- STAI
- SF-12
- PGI-S
- PGI-I

Patients were also asked about any doubts related to their diet and about the occurrence of any adverse event.

2.3.6 Weeks 18 and 24 visits

Anthropometric measurements were performed and the patients were asked to complete the following questionnaires:

- List of gluten-sensitivity symptoms
- FIQR
- PSQI
- BPI
- BDI-II
- STAI
- SF-12

- PGI-S
- PGI-I

Patients were also asked about the occurrence of any adverse event.

Patients who completed the study were asked to sign a form confirming their completion of the study.

2.4 Study Setting

This study was carried out at the Institute of Neuroscience at the University of Granada. Participants were provided with the appropriate dietary counseling by the investigators and a thorough evaluation of the patients' clinical status was conducted during each planned visit.

2.5 Randomization and blinding

The randomization sequence was computer-generated using the "QuickCalcs" from Graphpad Prism® for Windows, (http://www.graphpad.com/quickcalcs/RandMenu.cfm). The random number generator of this software is seeded with the time of day and each subject is first non-randomly assigned to a group, then the assignment of each subject is substituted with the group assignment of a randomly chosen subject. This entire process is repeated twice to ensure adequate randomization process (Suresh 2011).

After successfully fulfilling the eligibility criteria during the first visit, a second visit was scheduled during which participants were randomly assigned by an independent investigator, who was not involved neither in the evaluation nor in the selection of patients, to one of the two diet groups in accordance with the generated randomization list. Both patients and investigators, who were responsible for management, evaluation and data collection, were aware of the assigned diet.

2.6 Interventions

A dietary orientation session was scheduled with each participant following the random allocation to one of the two interventions. The study comprised an experimental intervention of a gluten-free diet and an active comparator intervention of a hypocaloric diet. A thorough explanation of the corresponding diet was ensured to reinforce the adequate adherence to the diet. A detailed reference document, which included a comprehensive explanation, of each diet was provided to every participant. Participants allocated to both interventions were instructed about the importance of a strict compliance to the diet as transgressions, whether voluntary or involuntary, could impair any potential benefit and represent a protocol violation.

Continuous follow-up on the participants' performance was ensured through the frequent investigator-patient interaction which ensured the clarification of any doubt related to the dietary therapy.

2.6.1 Gluten-free diet

This diet was not subject to any caloric restriction; elimination of gluten, in its various forms, was the only governing regulation. Participants were acquainted that gluten is a protein whose origin is specific cereals such as wheat, barley and rye. However, it has become a widely used ingredient in the food industry through the broad range of gluten-containing products. For instance, many packaged products include preservatives, thickeners, dyes, and flavorings derived from cereal derivatives such as wheat. The consumption of natural and fresh foods that do not contain gluten was recommended. Reading the "nutrition facts" label of the products was an important measure communicated to the participants and products lacking such label or generating doubts about the possible presence of gluten were to be avoided. Participants were informed about the availability of a wide variety of gluten-free products that substitute the prohibited gluten-containing ones. A detailed list of gluten-containing products, gluten-free products, and products that could be processed with gluten was supplied to the participants. The preparation of the gluten-free diet was to be carried out in a separate

or distinct place in order to avoid any possible contamination with the regular diet and participants were advised to use different utensils for preparing their gluten-free meals for the same reason.

2.6.2 Hypocaloric diet

Given the close correlation between obesity and fibromyalgia and the reported benefit of weight loss in fibromyalgia (Arranz et al. 2010), a hypocaloric diet was selected as active comparator. This diet consisted of multiple small-sized meals divided over five portions per day: breakfast, mid-day snack, lunch, afternoon snack and dinner. The meals were distributed in a way not to exceed a maximum intake of 1500 cal/day, and thus achieving a deficit diet convenient to ensure weight loss among participants. This intervention was delivered along with a detailed dietary program that included diversity of meal options corresponding to each designated portion along with the specific allowed quantities. Equivalent alternatives of same caloric intake for each designated portion are provided to the participants.

2.7 Assessment tools

Evaluation of the study subjects was conducted using the following assessment tools:

2.7.1 List of intestinal and extraintestinal gluten sensitivity related symptoms

A list of gluten sensitivity signs and symptoms, prepared by our investigation group, is presented in Table 3. This list included a total of 5 fibromyalgia-like symptoms and 14 intestinal and extraintestinal manifestations.

Table 3 List of gluten sensitivity symptoms

Fibromyalgia-like symptoms	Fatigue
	Depression
	Anxiety
	Sleep disturbances
	Memory problems
Gastrointestinal symptoms	Abdominal pain
	Bloating
	Diarrhea/constipation or alternation
	Lactose intolerance
	Alteration in the hepatic function[1]
	Nausea and/or vomiting
	Dyspepsia
	Pyrosis
	Steatorrhea
Extra-intestinal symptoms	Paresthesias
	Cutaneous lesions[2]
	Ankle edema
	Reproductive disorders[3]
	Anemia

1. Alterations in the normal hepatic function detected in previous analytical testing reported by the patient
2. Oral aphthous ulcers /dermatitis/frequent itching.
3. Late menarche/ irregular menstruation/early menopause/spontaneous abortions/fertility problems

Throughout the study, patients were evaluated for the presence of these symptoms. The total number of symptoms experienced at each visit was recorded for each patient. While analyzing the evolution of the total symptoms count, the following symptoms were included: fatigue, depression, anxiety, sleep problems, memory problems, abdominal problems, bloating, bowel changes, nausea and/or vomiting, dyspepsia, pyrosis, steatorrhea, paresthesias, cutaneous lesions, and ankle edema. The other symptoms were not counted as they were assumed to be slightly vulnerable to any change throughout the study period.

2.7.2 Anthropometric measurements

These measurements included the following:

- Body mass index (Kg/m^2): It is a measure of the body fat based on height and weight. It applies to both adult males and females. It is a reliable measure of body fatness and serves as an inexpensive alternative to direct measures of body fat. It is calculated by the dividing the weight in kilograms over the squared measure of height in meters (CDC 2015).

$$BMI = \frac{Weight(Kg)}{\left(Height(m)\right)^2}$$

The BMI is used to classify subjects into the following weight categories:

- < 18.5: Underweight
- 18.5 – 24.9: Normal
- 25.0 – 29.9: Overweight
- ≥30: Obese

- Waist circumference: The measurement of the waist circumference is mainly used to assess the abdominal fat. It is measured by placing the tape evenly around the abdomen at the level of the hip bone top (CDC 2015).

  Elevated waist circumference measures are linked to increased risk of obesity-related conditions. Higher risk of developing such conditions if:

  - Males> 102 cm
  - Females> 88 cm

- Chest circumference: It is the measure of the horizontal circumference around the thorax at the height of the fourth chondrosternal articulation (nipple level). The patient is asked to raise his arms and the tape is evenly placed around the breast keeping it perpendicular to the longitudinal axis of the body. The measurement is made under normal expiration.

2.7.3 Revised fibromyalgia impact questionnaire (FIQR)

It is a revised version of the original fibromyalgia impact questionnaire designed by Burckhardt et al. (Burckhardt et al. 1991). This original questionnaire was subject to several modifications with the FIQR being the most recent revision modified and validated by Bennett et al. (2009). This questionnaire is used to evaluate the health status of patients with fibromyalgia. In our investigation, we used the Spanish-validated version of the FIQR (Salgueiro et al. 2013).

It consists of a total of 21 questions distributed over the three domains: function (9 questions), overall impact (2 questions) and symptoms severity (10 questions). All questions are scored on a 0-10 visual analog scale. The total score of the FIQR is 100 (similar to the original FIQ) and is calculated by applying a normalization factor on the three items: The function domain is divided by three (30% contribution to the total score), the overall impact domain is divided by one (20% contribution to the total score), and the symptom severity domain is divided by two (50% contribution to the

total score). The summation of the normalized domains yields the total FIQR score. Higher scores correspond to greater global severity of fibromyalgia.

FIQR was associated with good psychometric properties (Bennett et al. 2009) which were also demonstrated in the Spanish-validated version (Salgueiro et al. 2013).

2.7.4 Pittsburgh sleep quality index (PSQI)

It is a self-rated questionnaire used to assess the sleep quality and disturbance over a 1-month period. It consists of 19 questions forming seven components: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction.

The global score of this test is calculated by adding the seven components scores. Each component score ranges between 0 and 3, yielding a global score that ranges between 0 and 21. Higher scores correspond to a poorer sleep quality. A global PSQI cutoff score of 5 is associated to 89.6% of diagnostic sensitivity and 86.5% of specificity (kappa = 0.75, $P < 0.001$) in distinguishing good and poor sleepers (Buysse et al. 1989). The Spanish-validated version of the PSQI was used (Royuela and Macias 1997).

2.7.5 Brief pain inventory-Short form (BPI)

It is a short self-administered questionnaire that was originally developed for use in cancer patients (Cleeland and Ryan 1994). Later, it has been also extensively used to evaluate pain in other chronic diseases. It is intended to assess the pain severity and its interference in the subject’s daily functioning.

It includes four items measuring the intensity of pain through a numerical scale ranging from 0 (no pain) to 10 (pain as bad as you can imagine). These four items measure the worst, least and average pain intensity during the last week in addition to the

current intensity of pain. The arithmetic mean of the response to these four items yields the intensity score.

The interference of pain is evaluated using seven items directed to different aspects of the daily activity including general activity, mood, walking ability, normal work, relations with other people, sleep and enjoyment of life. Each item is scored from 0 (Does not interfere) to 10 (Completely interferes). The score of the impact component is calculated from the arithmetic mean of the 7 items' answers.
The Spanish-validated version of the BPI was used (Badia et al. 2003).

2.7.6 Beck depression inventory-II (BDI-II)

It is a self-administered questionnaire that measures the severity of depressive symptoms. It consists of 21 questions directed to the different aspects of depression. Each item is scored from 0 to 3 leading to a total score that ranges between 0 and 63 (Beck et al. 1996). Two subcomponents scales can be obtained from the BDI-II including cognitive-affective component (12 items) and somatic component (9 items) (Dozois et al. 1998).

Depending on the total BDI-II score, patients are classified into:

- No or minimal depression: ≤ 13
- Mild depression: 14 – 19
- Moderate depression: 20 – 28
- Severe depression: >30 (Beck et al. 1996)

The Spanish-validated version of the BDI-II was used (Sanz Fernandez et al. 2003).

2.7.7 State-trait anxiety inventory (STAI)

It is a self-report questionnaire designed to measure the intensity of anxiety. It evaluates two distinct components: state anxiety (a temporary condition experienced in specific situations) and trait anxiety (a general tendency to perceive situations as

threatening) (Spielberger et al. 2002). It consists of 40 questions (20 questions for each component) that are scored on a 4-point Likert scale.

The 4-Likert scale answers of the state anxiety component include: 1: not at all, 2: somewhat, 3: moderately so, 4: very much so. On the other hand, the 4-Likert scale answers of the trait anxiety component include: 1: almost never, 2: sometimes, 3: often, 4: almost always.

Some of the items are worded positively, i.e., corresponding to less anxiety. Thus, after reversing these positively worded items, the score of each component is calculated ranging between 20 and 80 with higher scores indicating higher levels of anxiety.

2.7.8 Short-form health survey (SF-12)

It is an abbreviation of the SF-36 Health Survey used for the evaluation of health status and quality of life. It consists of 12 questions that are grouped to evaluate a physical component scale (PCS) and a mental components scale (MCS) (Ware et al. 1996).

Following the recalibration of the different items scores using specific regression weights, a total score is calculated for each component and then transformed into a 0 to 100 scale. Higher scores are indicative of a better quality of life.
The Spanish-validated version of the SF-12 was used (Vilagut et al. 2008).

2.7.9 Patient global impression scales of severity (PGI-S) and improvement (PGI-I)

They are two seven-Likert scales commonly used to measure the severity of a specific condition (PGI-S) and the patient's perception of the disease evolution following the initiation of therapy (PGI-I) (Guy et al. 1976).

The seven items of the PGI-S include: 1= normal (not at all ill); 2= borderline mentally ill; 3= mildly ill; 4= moderately ill; 5= markedly ill; 6= severely ill; and 7= among the most extremely ill.

The seven items of the PGI-I include: 1= very much improved; 2= much improved; 3= minimally improved; 4= no change; 5= minimally worse; 6= much worse; 7= very much worse.

2.7.10 Safety of the dietary interventions

Potential adverse events of each of the dietary interventions were collected by means of open-ended questions.

2.8 Primary and secondary outcome measures

Mean change, between baseline and the final visit, in the number of experienced gluten-sensitivity symptoms detailed in Table 3 constituted the primary outcome measure of our study.

Secondary outcome measures included the changes, between baseline and the final visit, in the BMI, FIQ-R, PSQI, BPI, BDI-II, STAI and SF-12, potential adverse events, PGI-S and PGI-I.

Additional secondary outcome measures included comparing the percentage of responders who achieved ≥20%, ≥30% or ≥50% in each of the primary and secondary variables.

2.9 Sample Size

The primary objective of the current study was to evaluate the improvement of the gluten sensitivity symptoms among patients with fibromyalgia. Given the absence of any validated scale that evaluates gluten sensitivity symptoms severity, we decided to carry out this study as pilot one, without a sample size calculation. We considered that it was

feasible to recruit an overall of 80 patients with fibromyalgia (40 patients are to be randomized to each group) over a two-year period.

2.10 Statistical Analyses

Baseline demographic and clinical characteristics of the participants of both groups were analyzed using descriptive statistics. Inferential statistics were employed to compare the effects of the two dietary interventions on the different outcome measures. Data were analyzed in a modified intention-to-treat (ITT) population; patients who complete at least one visit following diet initiation were included in the final analysis and missing values were imputed using the last observation carried forward (LOCF). For the continuous variables, the comparison between the two groups was conducted using student's t test. Analysis of Variance (ANOVA) was used to evaluate the significance of the participants' evolution within each group by comparing the differences of paired data scores at different time points compared to baseline. Fisher's exact test or chi-squared test will be used for analyzing qualitative outcome variables such as PGI and adverse events data. Paired proportions in each of the two groups were evaluated using McNemar test. A p-value <0.05 was considered statistically significant. Mean change from baseline was calculated for each continuous outcome measure in each of the two groups and mean differences between the two groups were reported along with a 95% confidence interval. Effect sizes were calculated using Cohen's formula and considered small when <0.5, medium when ranging between 0.5 to 0.79, and large when ≥ 0.8 (Kazis et al. 1989). Statistical analysis was performed using Graphpad Prism® 5 for Windows, Version 5.04 (GraphPad Software, San Diego California USA, www.graphpad.com).

3. Results

3.1 Subjects disposition

A total of 81 subjects were screened for eligibility, of whom 79 met the inclusion criteria. Seventy five out of the 79 subjects were randomly allocated to receive either GFD (n= 35) or HCD (n= 40) (Figure 3). These subjects constituted our ITT population. A total of 20 patients failed to complete the study, 11 (31%) from the GFD group and 9 (22.5%) from the HCD group (P= 0.4393). Reasons for drop-outs included loss of follow-up, protocol violation and lack of efficacy. Non-significant inter-group differences in any of the reasons for discontinuation were found.

3.2 Baseline clinical and demographic characteristics

The majority of the study participants were females (97%) with a mean age of 51.25±8.13. As shown in Table 4, comparable baseline demographic characteristics were seen in both groups with no significant statistical differences. With respect to the baseline clinical characteristics, as it can be seen in Table 5, no significant differences between the two groups were found excepting for the BMI (P= 0.0153) and waist circumference (P= 0.0093) that were higher in the HCD group. Patients, at baseline, showed severe fibromyalgia impact, sleep disturbances, depression, pain (severity and interference), anxiety (state and trait), and QOL disability (physical and mental).

Our study sample displayed a high frequency of comorbidites, whereby 89% of the total study population presented at least 4 comorbid conditions (91.4% in GFD and 87.5% in HCD). None of the subjects was “comorbid-free” in neither of the two groups. No significant differences between the two groups were found. The most frequent comorbidities included: osteoarthritis, anxiety-depressive disorders, headache, IBS, allergies and venous insufficiency (Table 6).

With respect to pharmacologic therapy, more than half (54.6%) of the total study population used at least four different medications (42.9% in GFD vs 65% in HCD). Only one patient in the GFD group was not taking any medication as compared to 3 patients in the HCD group. NSAIDs were among the most commonly prescribed medications

(82% in GFD vs 57.1% in HCD) followed by antidepressants (48.6% in GFD vs 82.5% in HCD) and sedative hypnotics (62.8% in GFD vs 57.5% in HCD) (Table 7).

Figure 3. Subjects Disposition

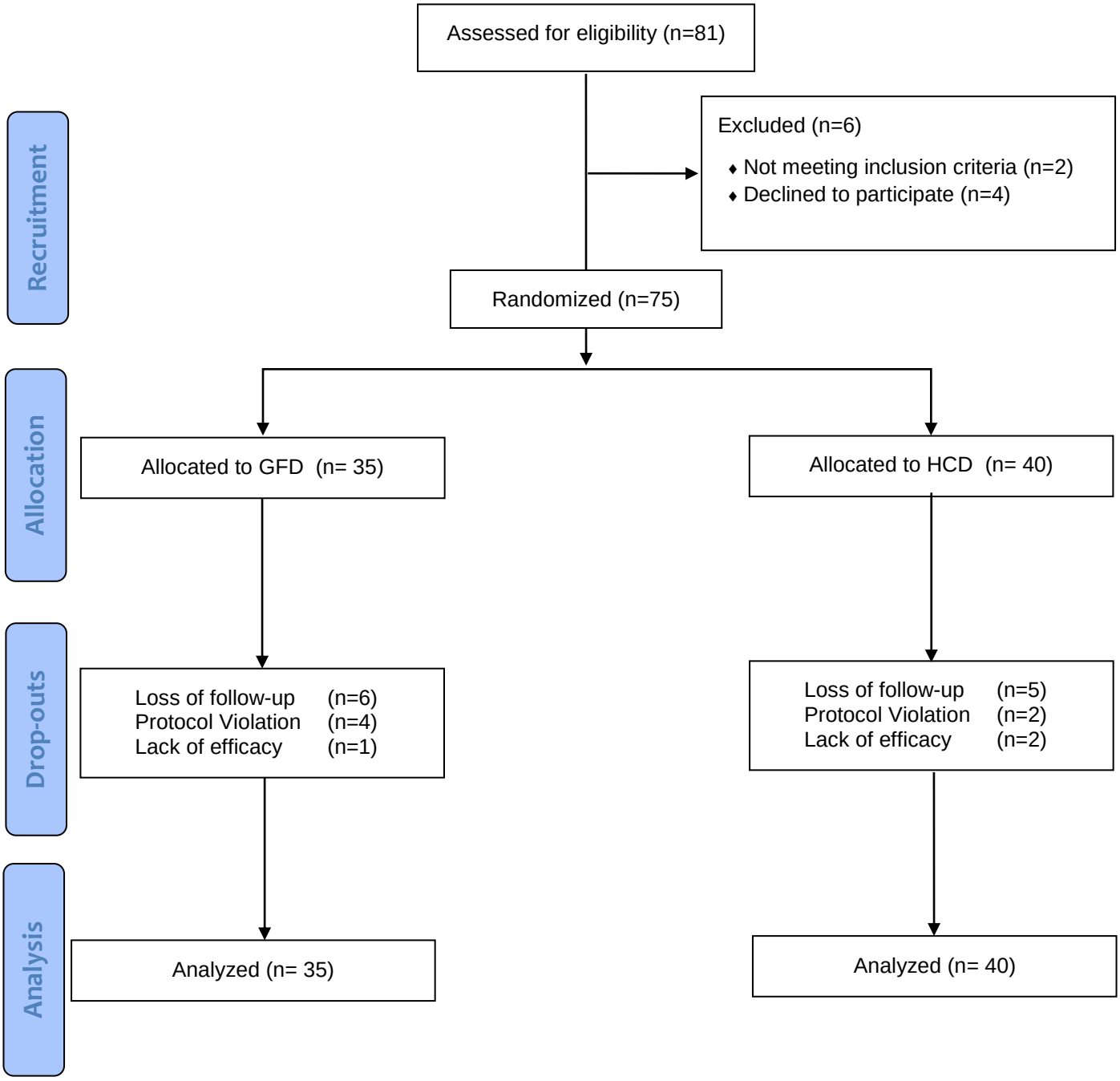


Table 4. Baseline demographic characteristics

	GFD (n=35)	**HCD (n= 40)**
Gender	**N (%)**	**N(%)**
F	35 (100)	38 (95)
M	0	2 (5)
Age		
(Mean±S.D.)	51.6 ± 7.34	50.9 ± 8.85
Educational Level		
No school	3 (8.6)	3 (7.5)
Primary	17 (48.6)	22 (55)
Secondary	14 (40)	13 (32.5)
University	0	1 (2.5)
Undisclosed	1 (2.8)	1 (2.5)
Occupation		
Work only at home	12 (34.3)	10 (25)
Work outside home	7 (20)	13 (32.5)
Sick leave	1 (2.8)	1 (2.5)
Disability	3 (8.6)	2 (5)
Unemployed	3 (8.6)	6 (15)
Retired	9 (25.7)	8 (20)
Toxic habits		
Smokers	8 (22.8)	9 (22.5)

Table 5. Baseline clinical characteristics

	GFD **Mean±S.D.**	**HCD** **Mean±S.D.**
Weight (kg)	69.9 ± 13.4	76.4 ± 13.7
Height (cm)	161.4 ± 6.3	159.2 ± 7.2
BMI (kg/m^2)	27.0 ± 5.85	30.2 ± 5.29
Chest Circumference (cm)	100.5 ± 16.9	102.9 ± 10.6
Waist Circumference (cm)	92.1 ± 14.1	99.4 ± 9.41
Gluten-sensitivity symptoms	12.6 ± 2.67	12.4 ± 2.13
Tender point count	17.2 ± 1.9	16.9 ± 2.5

Time since diagnosis (yrs)	5.9 ± 3.4	5.5 ± 4.1
Wide spread pain index	17.4 ± 2.2	16.8 ± 2.5
Symptom severity scale	10.3 ± 1.5	10.4 ± 1.6
FIQR	69.5 ± 16.3	70.4 ± 16.1
PSQI	15.5 ± 3.71	14.4 ± 3.83
BPI-Sev	6.67 ± 1.70	6.93 ± 1.42
BPI-Interf	7.13 ± 1.72	7.25 ± 1.56
BDI-II	30.5 ± 11.1	29.3 ± 11.4
STAI (State)	36.1 ± 11.2	35.3 ± 12.4
STAI (Trait)	39.7 ± 9.8	37.9 ± 10.7
SF-12 (PCS)	28.7 ± 4.70	27.1 ± 5.37
SF-12 (MCS)	31.90 ± 9.2	34.5 ± 12.2

Table 6. Comorbidities

	GFD N (%)	**HCD** N (%)
Comorbidities count		
≤3 Comorbidities	3 (8.6)	5 (12.5)
4-6 Comorbidities	11 (31.4)	20 (50)
≥7 Comorbidities	21 (60)	15 (37.5)
Otolaryngology		
Chronic pharyngitis	0	4 (10)
Hearing loss	5 (14.3)	5 (12.5)
Tinnitus	1 (2.85)	2 (5)
Vertigo	2 (5.7)	2 (5)
Cardiovascular		
Hypertension	5 (14.3)	10 (25)
Venous insufficiency	14 (40)	14 (35)
Respiratory		
Asthma	3 (8.6)	5 (12.5)
Recurrent pneumonia	0	2 (5)
Gastrointestinal		
IBS	10 (28.6)	10 (25)
Hemorrhoids	2 (5.7)	1 (2.5)
Hiatal Hernia	2 (5.7)	1 (2.5)
Peptic ulcer	2 (5.7)	1 (2.5)
Hepatobiliary		

Hepatitis A	3 (8.6)	1 (2.5)
Cholecystectomy	1 (2.8)	2 (5)
Renal		
Nephrolithiasis	7 (20)	5 (12.5)
Genitourinary		
Recurrent cystitis	3 (8.6)	2 (5)
Endometriosis	2 (5.7)	0
Endocrino-metabolic		
Diabetes Mellitus	5 (14.3)	4 (10)
Hypothyroidism	5 (14.3)	4 (10)
Dyslipidemia	4 (11.4)	2 (5)
Hyperthyroidism	2 (5.7)	0
Hematologic		
Anemia	8 (22.8)	3 (7.5)
Musculoskeletal		
Osteoarthritis	13 (37.1)	15 (37.5)
Osteopenia	5 (14.3)	2 (5)
Rheumatoid arthritis	2 (5.7)	2 (5)
Carpal Tunnel	3 (8.6)	2 (5)
Osteoporosis	3 (8.6)	5 (12.5)
*Other musculoskeletal disorders**	18 (51.4)	17 (42.5)
Dermatologic		
Atopic dermatitis	4 (11.4)	2 (5)
Urticaria	2 (5.7)	1 (2.5)

Photosensitivity	3 (8.6)	0
Neurologic		
Headache	8 (22.8)	6 (15)
Migraine	6 (17.1)	5 (12.5)
Trigeminal neuralgia	2 (5.7)	0
Polyneuropathy	2 (5.7)	0
Psychiatric		
Anxiety-Depressive disorders	17 (48.6)	15 (37.5)
Neoplasm		
Cervical dysplasia	2 (5.7)	0
Allergies	13 (37.1)	19 (47.5)

* calcification, canal stenosis, chondromalacia, epicondylitis, frequent fractures, herniation, scoliosis, spondylosis, tendinitis.

Table 7. Pharmacologic Therapy

	GFD N(%)	**HCD** N(%)
Medication use		
Medication-free	1 (2.8)	3 (7.5)
1-3 medications	13 (37.1)	9 (22.5)
≥4 medications	15 (42.9)	26 (65)
Analgesics		
Paracetamol	18 (51.4)	17 (42.5)
Tramadol	13 (37.1)	15 (37.5)
Other Opioids	2 (5.7)	3 (7.5)
Ibuprofen	10 (28.6)	8 (20)
Diclofenac	2 (5.7)	3 (7.5)
Dexketoprofen	5 (14.3)	1 (2.5)
Other NSAIDs	5 (14.3)	6 (15)
Metamizol	7 (20)	5 (12.5)
Antidepressants		
Amitriptyline	3 (8.6)	10 (25)
Cyclobenzaprine	1 (2.8)	1 (2.5)

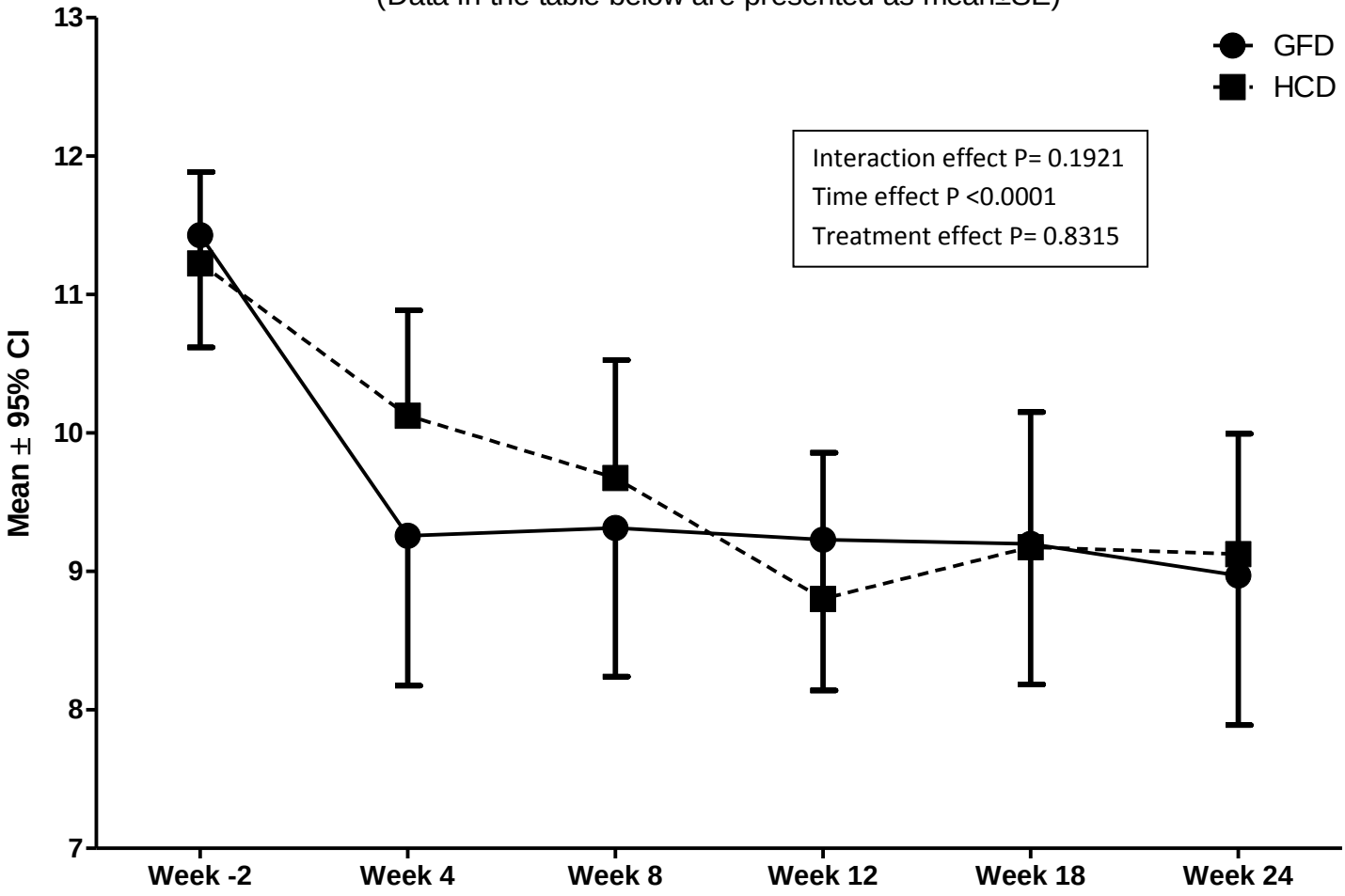
Duloxetine	4 (11.4)	7 (17.5)
Venlafaxine	2 (5.7)	1 (2.5)
Mirtazapine	1 (2.8)	0
SSRIs	4 (11.4)	8 (20)
Trazodone	2 (5.7)	6 (15)
Sedative-Hypnotics		
Benzodiazepines	18 (51.4)	19 (47.5)
Zolpidem	4 (11.4)	3 (7.5)
Buspirone	0	1 (2.5)
Anticonvulsants		
Pregabalin	7 (20)	9 (22.5)
Gabapentin	2 (5.7)	1 (2.5)
Topiramate	0	1 (2.5)
Atypical antipsychotics		
Quetiapine	2 (5.7)	2 (5)
Sulpiride	2 (5.7)	0
Antihypertensives	7 (20)	13 (32.5)
Antidiabetics	1 (2.8)	3 (7.5)
Antidyslipidemics	3 (8.6)	4 (10)
Levothyroxine	5 (14.3)	4 (10)
Proton pump inhibitors	19 (54.3)	12 (30)
Others		
Calcium	8 (22.8)	5 (12.5)
Bisphosphonates	4 (11.4)	1 (2.5)

3.3 Primary efficacy outcome measure

3.3.1 Changes in the total symptoms count between baseline and endpoint

An equivalent drop in the total number of gluten sensitivity symptoms, between baseline and endpoint, was seen in both groups (Figure 4). However, a more rapid decrease in the total count of these manifestations could be noticed in the GFD arm, whereby a sharp decrease from 11.43±0.43 at baseline to 9.26±0.53 on week 4 was recorded followed by an almost stable phase of total symptoms count (ranging between 8.9 and 9.23). On the other hand, patients in the HCD group experienced a gradual drop in the total number of gluten sensitivity symptoms; 11.22±0.33 to 8.8±0.52 between baseline and week 12 followed by a slight elevation to 9.17±0.48 and 9.12±0.43 on weeks 18 and 24, respectively. Two-way ANOVA for repeated measures demonstrated statistical significance with respect to time ($P<0.0001$), however interaction and treatment effects were non-significant.

Figure 4. Total number of symptoms between baseline and week 24
(Data in the table below are presented as mean±SE)

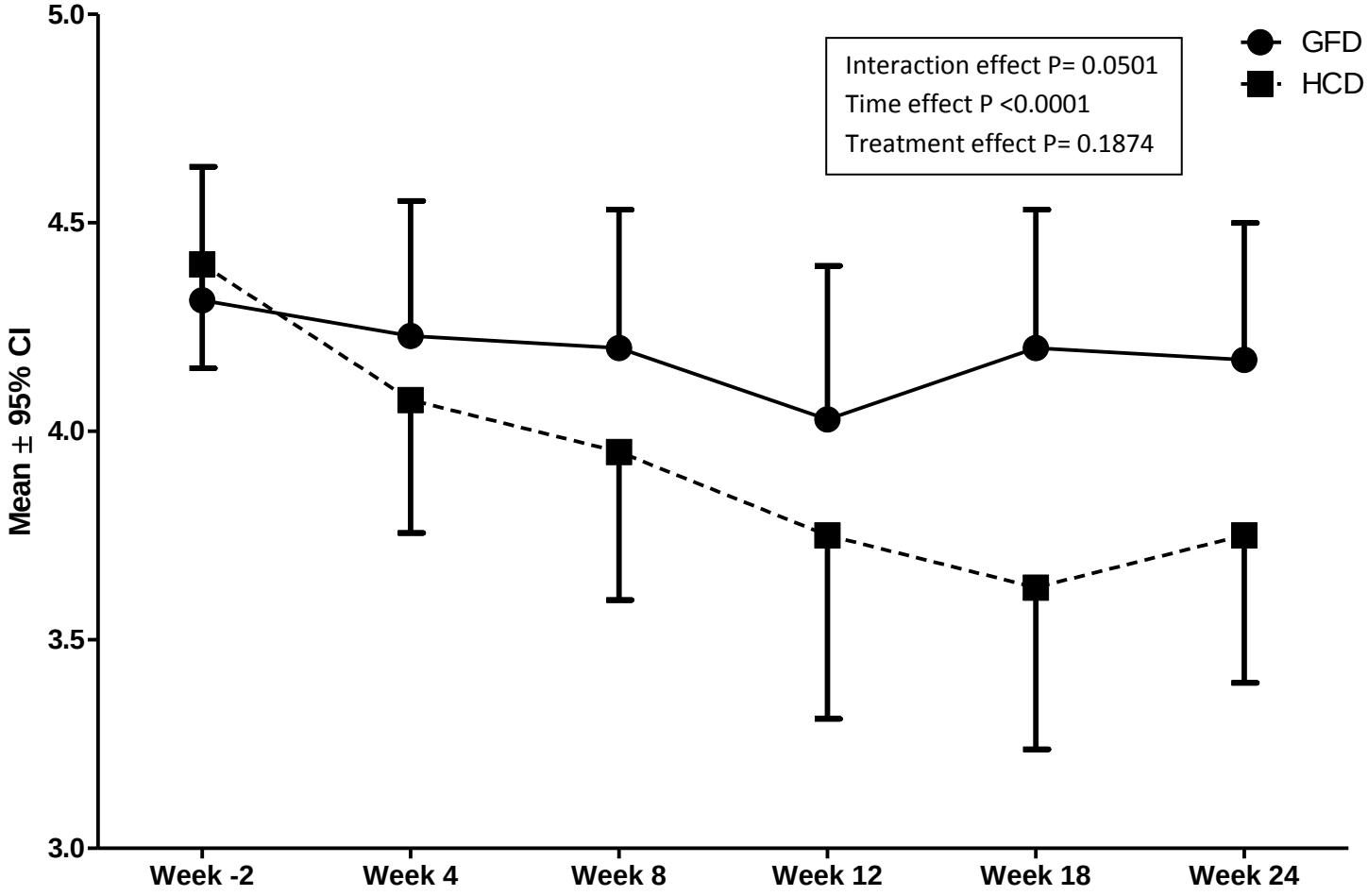


GFD	11.43±0.43	9.26±0.53	9.31±0.53	9.23±0.53	9.2±0.5	8.9±0.53
HCD	11.22±0.33	10.12±0.38	9.67±0.42	8.8±0.52	9.17±0.48	9.12±0.43

3.3.2 Changes in the subcomponents symptoms count between baseline and endpoint

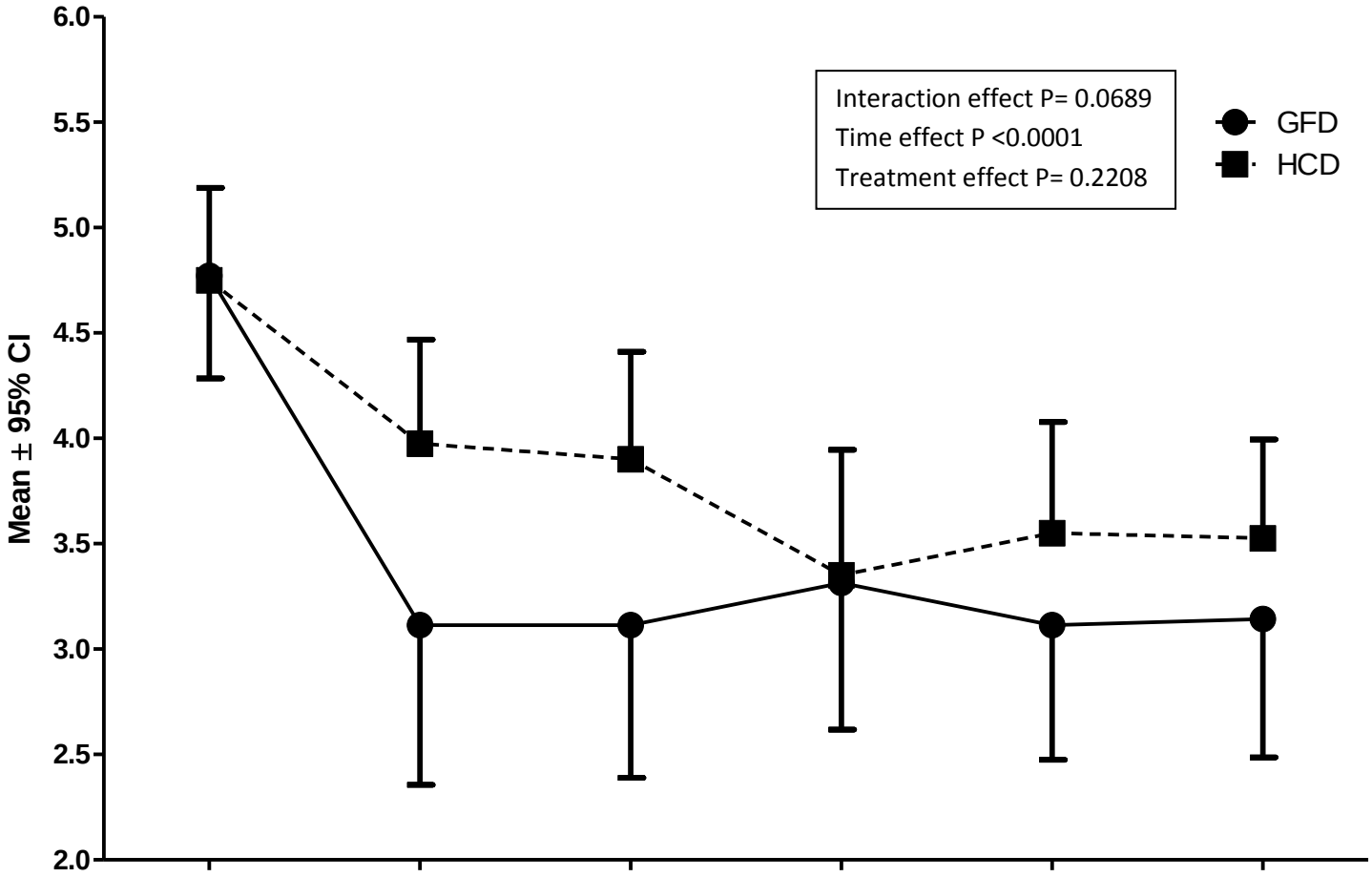
While a tendency of a better improvement in fibromyalgia-like symptoms could be noted with the HCD as compared to GFD (Figure 5), a greater tendency of improvement in GI and other symptoms (Figures 6 & 7) was reported by patients in the GFD group. However, the treatment effect did not reach statistical significance in any of the three subcomponents. Time-significant effect was seen in all of these manifestations ($P<0.0001$) and significant interaction effect was seen only with other symptoms count ($P= 0.0155$).

Figure 5. FM-like symptoms count between baseline and week 24
(Data in the table below are presented as mean±SE)



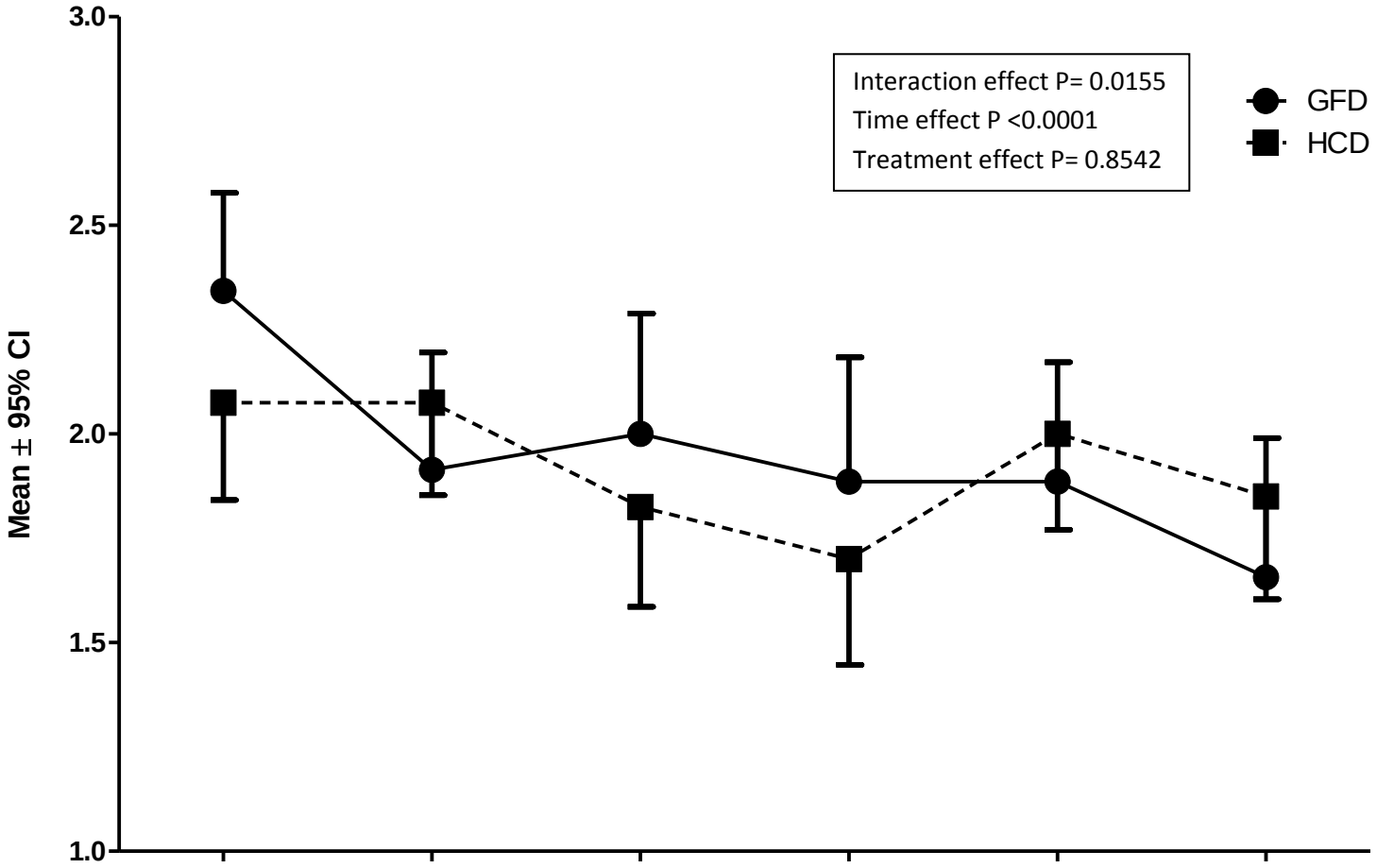
GFD	4.31±0.16	4.22±0.16	4.2±0.16	4.03±0.18	4.2±0.16	4.17±0.16
HCD	4.4±0.12	4.07±0.16	3.95±0.17	3.75±0.22	3.62±0.19	3.75±0.17

Figure 6. GI symptoms count between baseline and week 24
(Data in the table below are presented as mean±SE)



	Week -2	Week 4	Week 8	Week 12	Week 18	Week 24
GFD	4.7±0.24	3.11±0.37	3.11±0.36	3.31±0.34	3.11±0.31	3.14±0.32
HCD	4.75±0.22	3.97±0.24	3.9±0.25	3.35±0.29	3.55±0.26	3.52±0.23

Figure 7. Other symptoms count between baseline and visit 8
(Data in the table below are presented as mean±SE)



	Week -2	Week 4	Week 8	Week 12	Week 18	Week 24
GFD	2.34±0.11	1.91±0.14	2±0.14	1.89±0.15	1.89±0.14	1.66±0.16
HCD	2.07±0.11	2.07±0.11	1.82±0.12	1.7±0.12	2±0.11	1.85±0.12

3.3.3 Mean change difference in the gluten sensitivity symptoms count

Analyzing the difference of the mean change in the number of gluten sensitivity symptoms during each visit (Table 8), it can be noted the rapid drop in the total symptoms count on visit 4 in the GFD group which reached -2.2±0.47 as compared to -1.1±0.32 in the HCD group (mean difference -1.1; 95% CI -2.188, 0.04515). On this visit, statistically significant difference of change between the two groups was reached in the GI and other symptoms subcomponents.

On week 8, HCD started to demonstrate beneficial effects with a mean change in the total symptoms count from baseline reaching -1.55±0.41 as compared to -2.11±0.39 in the GFD group; mean difference of change between the two groups didn't reach statistical significance (mean difference -0.56; 95% CI -1.703, 0.5705). GFD preserved its superiority on week 8 with respect to the change in the GI symptoms count compared to HCD (mean difference -0.81; 95% CI -1.600, -0.01446).

On weeks 12, 18 and 24, the mean change in the total symptoms count from baseline was comparable in both groups with mean differences of 0.22, -0.18 and -0.36, respectively. Subcomponents symptoms count, by the end of study, revealed superiority of HCD in reducing fibromyalgia-like symptoms in contrast to GFD superiority in reducing other symptoms.

Table 8. Mean differences of change from baseline in the gluten sensitivity symptoms count on each visit

	GFD ***(mean change±SE)***	***HCD*** ***(mean change±SE)***	***Mean difference****: GFD-HCD* ***(95% CI)*** ***[P-value]***
Week 4			
Total number of symptoms	-2.2±0.47	-1.1±0.32	-1.1 (-2.19, 0.045) [0.0594]
Fibromyalgia-like symptoms	-0.08±0.13	-0.32±0.13	0.24 (-0.12, 0.60) [0.1946]
Gastrointestinal symptoms	-1.66±0.38	-0.77±0.22	**-0.88 (-1.73, -0.036) [0.0409]**
Other symptoms	-0.43±0.14	0±0.12	**-0.43 (-0.79, -0.068) [0.0204]**
Week 8			
Total number of symptoms	-2.11±0.39	-1.55±0.41	-0.56 (-1.70, 0.57) [0.3258]
Fibromyalgia-like symptoms	-0.11±0.18	-0.45±0.15	0.33 (-0.13, 0.8) [0.1524]
Gastrointestinal symptoms	-1.66±0.29	-0.85±0.27	**-0.81 (-1.6, -0.015) [0.0458]**
Other symptoms	-0.34±0.11	-0.25±0.15	-0.09 (-0.47, 0.29) [0.6272]
Week 12			
Total number of symptoms	-2.2±0.4	-2.4±0.42	0.22 (-0.96, 1.41) [0.7049]
Fibromyalgia-like symptoms	-0.28±0.15	-0.65±0.18	0.36 (-0.12, 0.85) [0.1376]
Gastrointestinal symptoms	-1.46±0.27	-1.4±0.27	-0.06 (-0.83, 0.71) [0.8825]
Other symptoms	-0.46±0.14	-0.37±0.14	-0.08 (-0.49, 0.32) [0.6867]
Week 18			
Total number of symptoms	-2.23±0.43	-2.05±0.41	-0.18 (-1.36, 1.0) [0.7644]
Fibromyalgia-like symptoms	-0.11±0.16	-0.77±0.19	**0.66 (0.16, 1.16) [0.0106]**
Gastrointestinal symptoms	-1.66±0.28	-1.2±0.25	-0.46 (-1.21, 0.29) [0.2275]
Other symptoms	-0.46±0.14	-0.07±0.12	**-0.38 (-0.75, -0.018) [0.0399]**
Week 24			
Total number of symptoms	-2.46±0.4	-2.1±0.38	-0.36 (-1.47, 0.75) [0.5232]
Fibromyalgia-like symptoms	-0.14±0.15	-0.65±0.18	**0.51 (0.038, 0.98) [0.0344]**
Gastrointestinal symptoms	-1.63±0.27	-1.2±0.24	-0.40 (-1.12, 0.32) [0.2667]
Other symptoms	-0.68±0.15	-0.22±0.13	**-0.46 (-0.85, -0.07) [0.0217]**

3.3.4 Changes in the percentage of patients experiencing gluten sensitivity symptoms

The evaluation of the number of patients experiencing gluten sensitivity symptoms in addition to the proportion change, between baseline and week 24, in each of these symptoms is shown in Table 9. With respect to fibromyalgia-like symptoms, the percentage of patients experiencing fatigue did not vary between baseline and week 24 in any group. Compared to GFD group, greater drop in the percentage of patients experiencing anxiety, sleep disorders and memory problems was seen in the HCD group; intra-group analysis revealed statistically significant change only in depression and anxiety in the HCD group.

Concerning gastrointestinal manifestations, a drop in the number of patients experiencing all these symptoms in both groups was observed. Both dietary interventions were associated with a significant intra-group decrease in the percentage of patients experiencing bloating, nausea/vomiting and pyrosis. While abdominal pain decreased significantly within HCD group, dyspepsia decreased significantly within GFD group. With respect to intergroup differences in the proportion of patients experiencing bloating, bowel changes or dyspepsia, a tendency of a better improvement in the GFD group as compared to HCD group was noted (Table 9).

With respect to other symptoms, significant intra-group decrease in the percentages of patients suffering from cutaneous lesions or ankle edema was seen in the GFD group. The proportion of patients experiencing cutaneous lesions significantly improved in the GFD group as compared to the HCD group.

Table 9. Change in the number of patients experiencing gluten-sensitivity symptoms

Symptom		Baseline N (%)	Week 24 N (%)	McNemar P-value	Difference in proportion change: GFD-HCD (95% CI)
Fatigue	GFD	35 (100)	34 (97.1)	1	0.029 (-0.03, 0.08)
	HCD	40 (100)	40 (100)	0.5	
Depression	GFD	26 (74.3)	22 (62.8)	0.125	-0.06 (-0.22, 0.10)
	HCD	28 (70)	21 (52.5)	**0.039**	
Anxiety	GFD	30 (85.7)	30 (85.7)	1	**-0.225 (-0.36, -0.09)**
	HCD	35 (87.5)	26 (65)	**0.022**	
Sleep disorders	GFD	31 (88.6)	30 (85.7)	1	**-0.146 (-0.28, -0.01)**
	HCD	35 (87.5)	28 (70)	0.065	
Memory problems	GFD	29 (82.8)	30 (85.7)	1	**-0.104 (-0.16, -0.04)**
	HCD	38 (95)	35 (87.5)	0.375	
Abdominal pain	GFD	28 (80)	21 (60)	0.065	-0.03 (-0.22, 0.16)
	HCD	32 (80)	23 (57.5)	**0.012**	
Bloating	GFD	34 (97.1)	22 (62.8)	**<0.001**	0.118 (-0.09, 0.32)
	HCD	40 (100)	31 (77.5)	**0.004**	
Bowel changes	GFD	27 (77.1)	21 (60)	0.109	0.096 (-0.06, 0.25)
	HCD	34 (85)	31 (77.5)	0.250	
Nausea/vomiting	GFD	20 (57.1)	8 (22.8)	**0.004**	0.068 (-0.14, 0.28)
	HCD	23 (57.5)	12 (30)	**0.007**	
Dyspepsia	GFD	31 (88.6)	22 (62.8)	**0.004**	0.158 (-0.02, 0.33)
	HCD	29 (72.5)	25 (62.5)	0.454	
Pyrosis	GFD	20 (57.1)	12 (34.3)	**0.039**	-0.017 (-0.21, 0.18)
	HCD	23 (57.5)	13 (32.5)	**0.006**	
Steatorrhea	GFD	7 (20)	4 (11.4)	0.508	0.011 (-0.11, 0.14)
	HCD	9 (22.5)	6 (15)	0.250	
Paresthesias	GFD	35 (100)	30 (85.7)	0.063	0.043 (-0.11, 0.19)
	HCD	40 (100)	36 (90)	0.125	
Cutaneous lesions	GFD	26 (74.3)	15 (42.8)	**0.001**	**0.285 (0.12, 0.45)**
	HCD	24 (60)	23 (57.5)	1	
Ankle edema	GFD	21 (60)	13 (37.1)	**0.021**	0.129 (-0.04, 0.3)
	HCD	19 (47.5)	15 (37.5)	0.344	

3.4 Secondary outcome measures

3.4.1 Anthropometric measures

Both dietary interventions were linked to improvements in the anthropometric measures (Table 10). GFD was associated with a drop in the BMI from 27±5.8 at baseline to 26.2±5.6 at endpoint; on the other hand, HCD led to a significant drop in the BMI from 30.2±5.3 to 29±5.3; effect sizes, however, were small. Two-way ANOVA for repeated measures demonstrated statistical significance with respect to time ($P<0.0001$) and treatment ($P= 0.0224$), however interaction was non-significant.

With respect to weight and chest circumference, the decrease in these outcome measures was greater in the HCD group as compared to the GFD group; however, results didn't reach statistical significance and, again, effect sizes were small.

Higher significant decrease, between baseline and endpoint, in the waist circumference was seen among patients on the HCD as compared to those on the GFD. Waist circumference decreased from 99.4±9.4 to 94.7±11.6 in the HCD group with a medium effect size, whereas, in the GFD group, it decreased from 91.7±14.4 to 90.4±13.5. Two-way ANOVA for repeated measures demonstrated statistical significance with respect to time ($P<0.0001$) and treatment ($P= 0.0323$), however interaction was non-significant.

The mean differences of change from baseline in anthropometric measures between the two groups are displayed in Table 11. Mean changes in the BMI were significantly higher among the HCD group only on weeks 8 and 12 with a mean change of 0.4330 (95% CI 0.06844, 0.7976) and 0.42 (95% CI 0.009281, 0.8358), respectively. Significantly higher decrease in the waist circumference was recorded on week 24 only with a mean difference of 3.32 (95% CI 0.4725, 6.178). Non-significant differences were seen in the remaining outcome measures across all the study visits.

Table 10. Anthropometric outcome measures

		Baseline Mean±S.D	Week 4 Mean±S.D	Week 8 Mean±S.D	Week 12. Mean±S.D	Week 18 Mean±S.D	Week 24 Mean±S.D	
BMI (Kg/m^2)	***GFD***	27±5.8	26.3±5.4	26.6±5.7	26.4±5.6	26.3±5.6	26.2±5.6	Interaction effect P= 0.1924
	ES		-0.12	-0.07	-0.10	-0.12	-0.14	**Time effect P <0.0001**
	HCD	30.2±5.3	29.6±5.2	29.3±5.2	29.2±5.3	29.1±5.3	29±5.3	**Treatment effect P= 0.0224**
	ES		-0.11	-0.17	-0.19	-0.21	-0.23	
Weight (Kg)	***GFD***	69.8±13.4	69.1±13.1	68.8±13.1	68.3±12.8	68.3±12.6	68±12.8	Interaction effect P= 0.0986
	ES		-0.05	-0.07	-0.11	-0.11	-0.13	**Time effect P <0.0001**
	HCD	76.5±13.7	74.9±13.3	74.3±13.5	74.1±13.9	73.6±13.8	73.5±13.8	Treatment effect P= 0.0663
	ES		-0.12	-0.16	-0.17	-0.21	-0.22	
Chest circumference (cm)	***GFD***	98.2±12.9	97.4±12.7	97±12.2	96.2±11.9	96.2±11.8	96.4±11.9	Interaction effect P= 0.8387
	ES		-0.06	-0.09	-0.15	-0.15	-0.14	**Time effect P <0.0001**
	HCD	102.9±10.6	101.7±10.9	100.7±10.7	100.4±10.3	100.4±10.1	100.6±10.6	Treatment effect P= 0.1034
	ES		-0.11	-0.21	-0.23	-0.23	-0.22	
Waist Circumference (cm)	***GFD***	91.7±14.4	91.9±14.4	90.8±13.7	90.8±14.4	90.1±14.1	90.4±13.5	Interaction effect P= 0.0448
	ES		0.01	-0.06	-0.06	-0.11	-0.09	**Time effect P <0.0001**
	HCD	99.4±9.4	98.2±10.3	97.5±10.9	96.4±10.4	95.9±10.2	94.7±11.6	**Treatment effect P= 0.0323**
	ES		-0.13	-0.20	-0.32	-0.37	**-0.5**	

Table 11. Mean differences of change from baseline in anthropometric measures

	GFD ***(Mean change±SE)***	***HCD*** ***(Mean change±SE)***	***Mean difference****: GFD-HCD* ***(95% CI)*** ***[Student's t-test P-value]***
Week 4			
BMI (Kg/m^2)	-0.68±0.4	-0.63±0.08	-0.04 (-0.82, 0.73) [0.9066]
Chest circumference (cm)	-0.82±0.5	-1.2±0.6	0.38 (-1.13, 1.89) [0.6133]
Waist circumference (cm)	0.14±0.7	-1.2±3.4	1.34 (-0.39, 3.07) [0.1259]
Week 8			
BMI (Kg/m^2)	-0.44±0.13	-0.87±0.12	**0.43 (0.07, 0.79) [0.0204]**
Chest circumference (cm)	-1.14±0.52	-2.22±0.63	1.08 (-0.58, 2.75) [0.1991]
Waist circumference (cm)	-0.86±0.74	-1.86±0.55	1 (-0.81, 2.82) [0.2736]
Week 12			
BMI (Kg/m^2)	-0.59±0.14	-1.02±0.14	**0.42 (0.001, 0.83) [0.0449]**
Chest circumference (cm)	-2±0.7	-2.5±0.5	0.52 (-1.14, 2.19) [0.5315]
Waist circumference (cm)	-0.9±0.9	-2.91±0.65	2 (-0.17, 4.19) [0.0698]
Week 18			
BMI (Kg/m^2)	-0.67±0.18	-1.12±0.19	0.45 (-0.07, 0.97) [0.0899]
Chest circumference (cm)	-2.01±0.54	-2.52±0.62	0.51 (-1.17, 2.19) [0.5452]
Waist circumference (cm)	-1.57±0.91	-3.43±0.84	1.87 (-0.61, 4.34) [0.1370]
Week 24			
BMI (Kg/m^2)	-0.75±0.2	-1.2±0.2	0.43 (-0.13, 1) [0.1317]
Chest circumference (cm)	-1.8±2.6	-2.4±4.5	0.61 (-1.12, 2.35) [0.4813]
Waist circumference (cm)	-1.3±0.8	-4.6±1.1	**3.32 (0.47, 6.18) [0.0228]**

3.4.2 FIQR

The effects of GFD and HCD on the global severity of fibromyalgia were similar as reflected by the FIQR results shown in Table 12. The adoption of GFD led to a decrease in the total FIQR scores from 69.5±16.3 at baseline to 60.3±19.6 at study endpoint. Similarly, the HCD decreased total FIQR scores from 70.4±16.1 to 61.7±22.2. Medium effect sizes were recorded by both dietary interventions by the end of the study. Two-way ANOVA for repeated measures demonstrated statistical significance with respect to time ($P<0.0001$), however treatment and interaction effects were non-significant.

With respect to the subscales of the FIQR, only time-significant changes were obtained within the three subscales. Both dietary interventions were associated with small effect sizes on the change of the function subscale. While GFD was associated with medium effect sizes on the change of both overall impact and symptoms severity scales, HCD had a medium effect size only on the change of the symptoms severity subscale. The outcomes of each of the 21 questions of the FIQR are also reported in Table 12.

Concerning the mean difference of change from baseline between the two groups in the total FIQR scores (Table 13), a more rapid drop in the total FIQR score was achieved in the GFD group on week 4 as compared to the HCD group (Mean difference: -1.3; 95% CI -7.762, 5.138). This was followed by the appearance of beneficial effects of HCD on week 8 making it superior to the GFD on weeks 8 and 12 with a mean difference of change reaching 1.2 (95% CI -5.929, 8.429) and 1.5 (95% CI -5.583, 8.589), respectively. In the last 2 visits, the mean difference of change in the total FIQR became equivalent for both groups. Differences between the two groups in the mean change of the total FIQR and its three subscales didn’t reach statistical significance on any of the study visits.

Table 12. FIQR total and subcomponents scores

		Baseline Mean±S.D	Week 4 Mean±S.D	Week 8 Mean±S.D	Week 12. Mean±S.D	Week 18 Mean±S.D	Week 24 Mean±S.D	
FIQR Total	*GFD*	69.5±16.3	64.3±19.8	64.8±18.7	64.1±16.9	61.4±18.2	60.3±19.6	Interaction effect P= 0.9464
	ES		-0.32	-0.29	-0.33	-0.49	**-0.56**	**Time effect P<0.0001**
	HCD	70.4±16.1	66.5±18.6	64.4±17.3	63.4±19.1	62.1±21.2	61.7±22.2	Treatment effect P= 0.8578
	ES		-0.24	-0.37	-0.43	**-0.51**	**-0.54**	
FIQR-Function	*GFD*	57.3±18.1	54.3±20.8	56.9±20.4	53.6±19.5	51.5±20.7	50.4±22	Interaction effect P= 0.5144
	ES		-0.16	-0.02	-0.20	-0.32	-0.38	**Time effect P= 0.0005**
	HCD	61.6±18.9	59.1±19.7	55.6±20.1	56.6±21	54.8±22.9	54.5±22.8	Treatment effect P= 0.4796
	ES		-0.13	-0.32	-0.29	-0.36	-0.37	
FIQR-Overall impact	*GFD*	13.7±5.2	12.1±5.4	11.3±5.9	11.7±5.9	10.8±6.1	10.9±6	Interaction effect P= 0.8748
	ES		-0.31	-0.46	-0.38	**-0.56**	**-0.54**	**Time effect P= 0.0004**
	HCD	12.9±5.4	12.3±5.5	12.1±4.8	11.9±5.4	10.8±5.4	10.7±5.7	Treatment effect P= 0.9827
	ES		-0.11	-0.15	-0.18	-0.39	-0.41	
FIQR-Symptoms severity	*GFD*	73.4±16.4	68.4±19.9	69±18.6	69.2±17.7	66.8±18.9	65.2±18.3	Interaction effect P= 0.6746
	ES		-0.30	-0.27	-0.25	-0.40	**-0.5**	**Time effect P<0.0001**
	HCD	73.9±15.3	69.1±18.2	67.6±17.3	65.4±17.4	66.2±20.3	65.2±20.4	Treatment effect P= 0.8357
	ES		-0.31	-0.41	-0.55	**-0.50**	**-0.57**	
1- Hair combing	*GFD*	4.7±3	4.6±3.1	4.9±2.9	4.4±2.9	4.2±2.9	4.1±3	Interaction effect P= 0.3142
	ES		-0.03	0.06	-0.1	-0.16	-0.2	Time effect P= 0.8061
	HCD	4.4±3.1	4.5±3.3	4.4±3.1	4.7±3.1	4.4±3.3	4.6±3.1	Treatment effect P= 0.9888
	ES		0.03	0	0.01	0	0.06	
2- Walking for 20 minutes	*GFD*	5.7±3.3	5.7±3.2	5.6±3.1	5.1±2.9	4.8±2.9	5.1±3.1	Interaction effect P= 0.6510
	ES		0	-0.0303	-0.18182	-0.27273	-0.18182	**Time effect P= 0.0021**
	HCD	6.3±3.2	5.7±3.3	5.6±3.1	5.7±3.1	5.2±3.2	5±5.4	Treatment effect P= 0.6513
	ES		-0.19	-0.22	-0.19	-0.34	-0.41	
3- Preparing a meal	*GFD*	5.4±2.8	5.1±2.6	5.1±2.8	4.9±2.8	4.8±2.7	4.7±2.9	Interaction effect P= 0.7324
	ES		-0.10714	-0.10714	-0.17857	-0.21429	-0.25	Time effect P= 0.1043
	HCD	5±3.1	5.1±2.8	4.3±3	4.7±3.1	4.4±3.1	4.5±3	Treatment effect P= 0.5829
	ES		0.03	-0.22	-0.01	-0.19	-0.16	
4- Cleaning floors	*GFD*	7.3±2.3	6.6±2.4	7.1±2.2	6.6±2.4	6.2±2.3	6.1±2.6	Interaction effect P= 0.4278
	ES		-0.30	-0.09	-0.30	-0.48	**-0.52**	**Time effect P<0.0001**
	HCD	7.6±2.2	6.9±3	6.5±2.7	6.5±2.4	6.5±2.6	6.3±2.7	Treatment effect P= 0.9177
	ES		-0.32	**-0.5**	**-0.5**	**-0.5**	**-0.59**	
5- Carrying a bag of	*GFD*	7.4±2.2	6.7±2.9	7.1±2.5	6.7±2.4	6.5±2.8	6.2±2.9	Interaction effect P= 0.5579

groceries	***ES***		-0.32	-0.14	-0.32	-0.41	**-0.54**	**Time effect P= 0.0013**
	HCD	8.2±1.8	7.7±2.4	7.2±2.2	7.5±2.4	7.3±2.5	7.1±2.6	Treatment effect P= 0.1122
	ES		-0.28	**-0.55**	-0.39	**-0.5**	**-0.61**	
6- Climbing one flight of stairs	*GFD*	7.4±2.1	6.9±2.6	7.4±2.3	7.1±2.6	6.5±2.6	6.4±2.7	Interaction effect P= 0.4593
	ES		-0.24	0	-0.14	-0.43	-0.48	**Time effect P<0.0001**
	HCD	8.2±2.1	8±1.9	7.5±2.3	7.4±2.3	7.1±2.6	7±2.7	Treatment effect P= 0.2005
	ES		-0.09	-0.33	-0.38	**-0.52**	**-0.57**	
7- Changing bed sheets	*GFD*	6.3±2.8	6.1±2.8	6.2±2.8	5.7±2.7	5.5±2.7	5.7±2.9	Interaction effect P= 0.8332
	ES		-0.07	-0.036	-0.21	-0.28	-0.21	**Time effect P= 0.0087**
	HCD	7.2±2.9	6.9±3	6.5±3	6.4±2.8	6.5±2.9	6.3±2.9	Treatment effect P= 0.2281
	ES		-0.10	-0.24	-0.27	-0.24	-0.31	
8- Sitting in a chair for 45 mins	*GFD*	6.8±3	6.4±3.2	6.9±2.9	6.5±3	6.4±3	5.8±3.3	Interaction effect P= 0.3078
	ES		-0.13	0.03	-0.1	-0.13	-0.33	Time effect P= 0.2865
	HCD	7.2±2.8	7.2±2.7	6.8±3	7±2.7	6.7±3.1	7±2.9	Treatment effect P= 0.3783
	ES		0	-0.14	-0.07	-0.18	-0.07	
9- Shopping for groceries	*GFD*	6.2±2.9	6.2±2.6	6.6±2.6	6.1±2.8	6.4±2.8	6.3±2.5	Interaction effect P= 0.2674
	ES		0	0.14	-0.03	0.07	0.03	Time effect P= 0.5764
	HCD	7.4±2.4	7±2.5	6.6±2.6	6.6±2.7	6.5±2.8	6.5±2.6	Treatment effect P= 0.3336
	ES		-0.17	-0.33	-0.33	-0.37	-0.37	
10- FM preventing me accomplishing goals	*GFD*	7.2±2.4	6±3	5.6±3	5.8±2.9	5.4±3.2	5.7±6	Interaction effect P= 0.3893
	ES		**-0.5**	**-0.67**	**-0.58**	**-0.75**	**-0.62**	Time effect P= 0.1059
	HCD	6.3±2.8	6.1±2.8	6.8±7.8	5.9±2.7	5.5±2.6	5.4±3.1	Treatment effect P= 0.9248
	ES		-0.07	0.18	-0.14	-0.28	-0.32	
11- Overwhelmed by FM	*GFD*	6.5±3	6±2.8	5.7±3.1	5.8±3.1	5.4±3.1	5.2±3.2	Interaction effect P= 0.8885
	ES		-0.17	-0.27	-0.23	-0.37	-0.43	**Time effect P= 0.0004**
	HCD	6.6±3.2	6.2±3.1	6.4±2.7	6±2.9	5.3±3	5.2±3.1	Treatment effect P= 0.7482
	ES		-0.12	-0.06	-0.19	-0.41	-0.44	
12- Pain	*GFD*	7.7±1.6	7.3±2	7.2±1.8	7.4±2.1	7±2.2	6.7±2.2	Interaction effect P= 0.6569
	ES		-0.25	-0.31	-0.19	-0.44	**-0.62**	**Time effect P<0.0001**
	HCD	8±1.7	7.2±2.1	6.8±2.1	7±2.1	6.8±2.5	6.6±2.5	Treatment effect P= 0.7470
	ES		-0.47	**-0.70**	**-0.59**	**-0.71**	**-0.82**	
13- Energy	*GFD*	7.7±2.4	6.7±2.9	7.2±2.3	7.4±2.1	6.6±2.7	6.5±2.7	Interaction effect P= 0.2291
	ES		-0.42	-0.21	-0.12	-0.46	**-0.5**	**Time effect P= 0.004**
	HCD	8±2.1	7.6±2	7.3±2.2	7±2.4	7.1±2.6	7.1±2.4	Treatment effect P= 0.4336
	ES		-0.19	-0.33	-0.48	-0.43	-0.43	
14- Stiffness	*GFD*	7±2.2	7.1±2.1	7±2.1	6.7±2.5	6.2±2.7	5.7±3	Interaction effect P= 0.2337
	ES		0.045	0	-0.14	-0.36	**-0.59**	**Time effect P<0.0001**

	HCD	7.5±1.8	6.7±2.6	6.8±2.5	6.6±2.3	6.3±2.8	6.5±2.6	Treatment effect P= 0.8217
	ES		-0.44	-0.39	**-0.5**	**-0.67**	**-0.55**	
15- Sleep	*GFD*	8.7±1.9	8±2.1	7.6±2.3	7.6±2.7	7.6±2.3	7.4±2.5	Interaction effect P= 0.9198
	ES		-0.37	**-0.58**	**-0.58**	**-0.58**	**-0.68**	**Time effect P= 0.0004**
	HCD	8.2±2.5	7.9±2.4	7.8±2.3	7.3±2.5	7.5±2.6	7.2±2.9	Treatment effect P= 0.7105
	ES		-0.12	-0.16	-0.36	-0.28	-0.4	
16- Depression	*GFD*	7.2±2.6	6.3±3.3	6.5±3	6.1±3.2	6±3.1	6.1±3	Interaction effect P= 0.9215
	ES		-0.35	-0.27	-0.42	-0.46	-0.42	**Time effect P= 0.0037**
	HCD	6.1±3.3	5.1±3.2	5.1±3	5.2±2.7	5.2±3.2	4.9±3.2	Treatment effect P= 0.0774
	ES		-0.30	-0.30	-0.27	-0.27	-0.36	
17- Memory problems	*GFD*	7±2.4	7±2.7	6.5±2.9	7±2.4	6.6±2.5	6.8±2.7	Interaction effect P= 0.6668
	ES		0	-0.20833	0	-0.16667	-0.08333	Time effect P= 0.3807
	HCD	7.2±2.3	7.1±2.5	7.1±2.5	6.7±2.7	6.7±2.9	7±2.9	Treatment effect P= 0.7927
	ES		-0.04	-0.04	-0.22	-0.22	-0.09	
18- Anxiety	*GFD*	7.4±2.6	6.7±2.9	7.2±2.5	6.7±2.8	6.9±2.5	6.6±2.8	Interaction effect P= 0.5945
	ES		-0.27	-0.08	-0.27	-0.19	-0.31	Time effect P= 0.2159
	HCD	6.3±3.4	5.9±3.3	5.7±3.2	5.5±3	5.7±3.3	6±3.4	Treatment effect P= 0.0835
	ES		-0.12	-0.18	-0.23	-0.18	-0.09	
19- Tenderness to touch	*GFD*	7.2±2.4	6.7±3.2	6.7±2.5	7.3±2.2	7±2.5	7.1±2.4	Interaction effect P= 0.0974
	ES		-0.21	-0.21	0.04	-0.08	-0.04	Time effect P= 0.1805
	HCD	7.7±2.1	7.1±2.1	7.3±2	6.9±2.1	7.1±2.1	6.5±2.6	Treatment effect P= 0.7666
	ES		-0.28	-0.19	-0.38	-0.28	**-0.57**	
20- Balance problems	*GFD*	6.5±3.1	6.1±3.2	5.7±3	6.1±3.2	5.7±3.2	5.8±3.1	Interaction effect P= 0.9213
	ES		-0.13	-0.26	-0.13	-0.26	-0.22	**Time effect P= 0.0015**
	HCD	7.4±2.5	6.9±2.5	6.7±2.6	6.6±2.6	6.5±2.6	6.4±2.7	Treatment effect P= 0.1928
	ES		-0.2	-0.28	-0.32	-0.36	-0.4	
21- Sensitivity to loud	*GFD*	7±2.8	6.3±3.1	7.2±2.7	6.8±2.8	6.8±2.6	6.4±2.6	Interaction effect P= 0.0865
noises, bright light, odors	***ES***		-0.25	0.07	-0.07	-0.07	-0.21	Time effect P= 0.4030
and cold	*HCD*	7.3±2.5	7.3±2.3	6.9±2.4	6.6±2.6	7±2.3	6.9±2.4	Treatment effect P= 0.6597
	ES		0	-0.16	-0.28	-0.12	-0.16	

Table 13. Mean differences of change from baseline in FIQR total and its subscales scores

	GFD ***(Mean change±SE)***	***HCD*** ***(Mean change±SE)***	***Mean difference****: GFD-HCD* ***(95% CI)*** ***[Student's t-test P-value]***
Week 4			
FIQR-Total	-5.2±1.7	-3.8±2.6	-1.3 (-7.76, 5.14) [0.6859]
Function score	-3±2.5	-2.5±2.9	-0.42 (-8.23, 7.38) [0.9145]
Overall impact score	-1.6±0.7	-0.6±0.9	-1.03 (-3.36, 1.29) [0.3788]
Symptom severity score	-5±1.8	-4.7±2.3	-0.28 (-6.29, 5.73) [0.9266]
Week 8			
FIQR-Total	-4.7±2.2	-6±2.8	1.2 (-5.93, 8.43) [0.7292]
Function score	-0.43±2.9	-6±2.9	5.62 (-6.05, 2.89) [0.1747]
Overall impact score	-2.4±0.8	-0.8±1	-1.57 (-4.26, 1.12) [0.2481]
Symptom severity score	-4.4±2	-6.3±2.5	1.89 (-4.7, 8.49) [0.5681]
Week 12			
FIQR-Total	-5.4±2.1	-6.9±2.8	1.5 (-5.58, 8.59) [0.6734]
Function score	-3.7±2.9	-5±2.8	1.31 (-6.83, 9.44) [0.7494]
Overall impact score	-2±0.8	-1±1.1	-1.02 (-3.88, 1.82) [0.4737]
Symptom severity score	-4.1±2.1	-8.5±2.5	4.38 (-2.25, 11.02) [0.1916]
Week 18			
FIQR-Total	-8.1±1.9	-8.2±3.1	0.1 (-7.41, 7.66) [0.9738]
Function score	-5.8±2.7	-6.8±3.2	1 (-7.49, 9.48) [0.8148]
Overall impact score	-2.9±1	-2.1±1	-0.78 (-3.69, 2.13) [0.5919]
Symptom severity score	-6.6±1.9	-7.7±2.9	1.15 (-6.04, 8.35) [0.7499]
Week 24			

	GFD ***(Mean change±SE)***	***HCD*** ***(Mean change±SE)***	***Mean difference****: GFD-HCD* ***(95% CI)*** ***[Student's t-test P-value]***
FIQR-Total	-9.2±2	-8.7±3.2	-0.5 (-8.25, 7.24) [0.8969]
Function score	-6.9±2.7	-7.1±3.1	0.24 (-8.07, 8.55) [0.9543]
Overall impact score	-2.8±0.9	-2.2±1	-0.6 (-3.39, 2.19) [0.6696]
Symptom severity score	-8.1±2.1	-8.7±2.9	0.53 (-6.84, 7.91) [0.8859]

3.4.3 PSQI

Similar to previous outcome measures, the effect of GFD on sleep disturbances was characterized by a faster pattern of improvement as compared to HCD, whereby PSQI total score decreased from 15.5±3.7 at baseline to 13.7±4.1 on week 4 achieving an effect size of 0.48; on the other hand, the HCD led to a very slight decrease in the total PSQI score from 14.4±3.8 at baseline to 14.1±3.8 on week 4 with non-substantial effect size of 0.07 (Table 14). In the following visits, the beneficial effect of GFD was maintained reaching a medium effect size of 0.5 on week 18, however, a partial loss of this effect was seen on week 24 when the total PSQI score increased again to 14.5±3.7. With respect to HCD, a gradual decrease in the total PSQI score was achieved reaching 13.6±4.5 on week 12 which was maintained stable until the end of the study. Only time significant changes were obtained (P= 0.0058); treatment and interaction effects were non-significant.

Subcomponent analysis of the PSQI revealed that the improvement of the total PSQI scores was attributed to the subjective sleep quality component that was associated with greater effect sizes in the GFD group as compared to the HCD group. The effects of both dietary interventions on the remaining subcomponents were small.

Comparing the mean differences of change from baseline between the two groups on the total PSQI score confirmed the faster effect achieved by GFD in improving sleep disturbances as a mean difference of -1.5 (95% -3.041, 0.04053) was seen on week 4 (Table 15). This difference, although non-statistically significant, was attributed to the subjective sleep quality subcomponent where a significant difference in the mean change of this subcomponent was noted (Mean difference: -0.4; 95% CI -0.7419, -0.001). The mean difference of change in the total PSQI between the two groups was maintained on week 8; however, the subcomponents responsible for this difference were the combination of subjective sleep quality, sleep duration and habitual sleep efficiency. A decrease in the mean differences of change between the two groups on

the total PSQI score was seen in the subsequent visits, reaching -0.2 (-2.081, 1.617) on week 24.

Table 14. PSQI total and subcomponent scores throughout the study

		Baseline Mean±S.D	Week 4 Mean±S.D	Week 8 Mean±S.D	Week 12. Mean±S.D	Week 18 Mean±S.D	Week 24 Mean±S.D	
PSQI-Total	*GFD*	15.5±3.7	13.7±4.1	13.9±3.9	14.1±3.6	13.6±3.9	14.5±3.7	Interaction effect P= 0.1736
	ES		-0.49	-0.43	-0.38	-0.51	-0.27	**Time effect P= 0.0058**
	HCD	14.4±3.8	14.1±3.8	14.2±3.7	13.6±4.5	13.6±4.3	13.6±4.5	Treatment effect P= 0.6913
	ES		-0.08	-0.05	-0.21	-0.21	-0.21	
Subjective sleep quality	*GFD*	2.3±0.6	1.8±0.7	1.8±0.8	1.9±0.7	1.8±0.8	1.9±0.8	Interaction effect P= 0.1673
	ES		**-0.83**	**-0.83**	**-0.67**	**-0.83**	**-0.67**	**Time effect P< 0.0001**
	HCD	2.1±0.9	1.9±0.8	1.9±0.7	1.8±0.9	1.8±0.8	1.7±0.9	Treatment effect P= 0.8802
	ES		-0.22	-0.22	-0.33	-0.33	-0.44	
Sleep latency	*GFD*	2.2±0.9	2.1±0.9	2±0.9	2±0.8	1.9±0.9	2.2±0.9	Interaction effect P= 0.5470
	ES		-0.11	-0.22	-0.22	-0.33	0	**Time effect P= 0.0268**
	HCD	2.2±0.8	1.9±0.9	2.1±0.8	2±0.9	1.8±0.9	1.9±0.9	Treatment effect P= 0.6303
	ES		-0.37	-0.12	-0.25	-0.5	-0.37	
Sleep duration	*GFD*	2.1±1	1.8±1	1.7±1	1.7±0.9	1.8±1.1	1.8±1	Interaction effect P= 0.2952
	ES		-0.3	-0.4	-0.4	-0.3	-0.3	Time effect P= 0.1678
	HCD	1.8±1	1.8±1	1.8±1	1.8±1	1.8±1	1.7±1	Treatment effect P= 0.6882
	ES		0	0	0	0	-0.1	
Habitual sleep efficiency	*GFD*	2.2±1.1	1.9±1.1	1.8±1.2	1.8±1.2	1.7±1.2	2±1.1	Interaction effect P= 0.4657
	ES		-0.27	-0.36	-0.36	-0.45	-0.18	Time effect P= 0.0768
	HCD	1.9±1.3	1.8±1.3	1.9±1.2	1.7±1.3	1.8±1.1	1.8±1.3	Treatment effect P= 0.6596
	ES		-0.08	0	-0.15	-0.08	-0.08	
Sleep disturbances	*GFD*	2.2±0.6	2.2±0.7	2.3±0.7	2.2±0.6	2.2±0.7	2.2±0.7	Interaction effect P= 0.6915
	ES		0	0.17	0	0	0	Time effect P= 0.4052
	HCD	2.2±0.5	2.3±0.6	2.3±0.5	2.2±0.6	2.1±0.7	2.2±0.7	Treatment effect P= 0.9627
	ES		0.2	0.2	0	-0.2	0	
Use of sleeping medication	*GFD*	2±1.2	1.9±1.3	2±1.2	2.3±1.2	2±1.3	2.2±1.2	Interaction effect P= 0.2122
	ES		-0.08	0	0.25	0	0.17	Time effect P= 0.4750
	HCD	1.9±1.4	2±1.3	1.9±1.3	1.9±1.4	2.1±1.3	2.1±1.3	Treatment effect P= 0.7602
	ES		0.07	0	0	0.14	0.14	
Daytime dysfunction	*GFD*	2.3±0.8	2±0.8	2.2±0.8	2.2±0.7	2.1±0.8	2.1±0.8	Interaction effect P= 0.5991
	ES		-0.37	-0.12	-0.12	-0.25	-0.25	Time effect P= 0.6820
	HCD	2.2±0.8	2.3±0.8	2.2±0.8	2.2±0.8	2.1±0.9	2.1±0.8	Treatment effect P= 0.7625
	ES	0.12	0	0	-0.12	-0.12	-0.12	

Table 15. Mean changes in PSQI total and subcomponent scores from baseline

	GFD ***(Mean change±SE)***	***HCD*** ***(Mean change±SE)***	***Mean difference****: GFD-HCD* ***(95% CI)*** ***[Student's t-test P-value]***
Week 4			
PSQI-Total	-1.8±0.4	-0.3±0.6	-1.5 (-3.04, 0.04) [0.0559]
Subjective sleep quality	-0.6±0.1	-0.2±0.1	-0.4 (-0.74, -0.0001) [**0.0492**]
Sleep latency	-0.17±0.1	-0.22±0.1	0.05 (-0.36, 0.47) [0.7991]
Sleep duration	-0.3±0.1	-0.02±0.1	-0.3 (-0.66, 0.14) [0.2030]
Habitual sleep efficiency	-0.3±0.1	-0.1±0.2	-0.2 (-0.72, 0.29) [0.3996]
Sleep disturbances	-0.06±0.1	0.1±0.1	-0.16 (-0.46, 0.15) [0.3046]
Use of sleeping medication	-0.17±0.2	0.07±0.2	-0.24 (-0.80, 0.31) [0.3793]
Daytime dysfunction	-0.22±0.1	0.07±0.2	-0.3 (-0.75, 0.14) [0.1813]
Week 8			
PSQI-Total	-1.6±0.4	-0.2±0.6	-1.43 (-2.86, 0.002) [0.0501]
Subjective sleep quality	-0.5±0.1	-0.2±0.1	-0.3 (-0.68, 0.058) [0.0969]
Sleep latency	-0.25±0.1	-0.1±0.1	-0.15 (-0.54, 0.23) [0.4160]
Sleep duration	-0.4±0.1	0.02±0.2	-0.4 (-0.89, 0.02) [0.0599]
Habitual sleep efficiency	-0.4±0.2	-0.02±0.2	-0.4 (-0.97, 0.17) [0.1626]
Sleep disturbances	0.08±0.1	0.07±0.1	0.01 (-0.27, 0.29) [0.9401]
Use of sleeping medication	-0.028±0.2	-0.025±0.2	-0.003 (-0.60, 0.59) [0.9906]
Daytime dysfunction	-0.1±0.1	0.02±0.2	-0.1 (-0.55, 0.27) [0.5036]
Week 12			
PSQI-Total	-1.4±0.4	-0.8±0.7	-0.6 (-2.35, 1.15) [0.4970]
Subjective sleep quality	-0.4±0.1	-0.3±0.2	-0.1 (-0.57, 0.36) [0.6609]
Sleep latency	-0.25±0.1	-0.17±0.1	-0.08 (-0.49, 0.32) [0.6877]
Sleep duration	-0.4±0.1	-0.07±0.1	-0.33 (-0.79, 0.08) [0.1107]

	GFD ***(Mean change±SE)***	***HCD*** ***(Mean change±SE)***	***Mean difference****: GFD-HCD* ***(95% CI)*** ***[Student's t-test P-value]***
Habitual sleep efficiency	-0.4±0.2	-0.2±0.2	-0.2 (-0.81, 0.35) [0.4281]
Sleep disturbances	0±0.1	0.05±0.1	-0.05 (-0.34, 0.24) [0.7367]
Use of sleeping medication	0.23±0.2	-0.07±0.2	-0.3 (-0.29, 0.89) [0.3116]
Daytime dysfunction	-0.06±0.1	0.02±0.2	-0.08 (-0.48, 0.32) [0.6835]
Week 18			
PSQI-Total	-2±0.5	-0.8±0.7	-1.2 (-3.03, 0.64) [0.1969]
Subjective sleep quality	-0.5±0.1	-0.3±0.2	-0.2 (-0.68, 0.2) [0.2773]
Sleep latency	-0.28±0.1	-0.32±0.1	0.04 (-0.37, 0.45) [0.8494]
Sleep duration	-0.37±0.1	-0.02±0.2	-0.35 (-0.79, 0.10) [0.1284]
Habitual sleep efficiency	-0.5±0.2	-0.1±0.2	-0.4 (-1.0, 0.22) [0.1950]
Sleep disturbances	-0.03±0.1	-0.07±0.1	0.04 (-0.28, 0.38) [0.7811]
Use of sleeping medication	-0.06±0.2	0.12±0.2	-0.18 (-0.79, 0.42) [0.5514]
Daytime dysfunction	-0.14±0.1	-0.07±0.2	-0.07 (-0.52, 0.38) [0.7639]
Week 24			
PSQI-Total	-1±0.5	-0.8±0.7	-0.2 (-2.08, 1.62) [0.8029]
Subjective sleep quality	-0.4±0.1	-0.4±0.2	0 (-0.45, 0.45) [1]
Sleep latency	-0.06±0.1	-0.23±0.1	0.17 (-0.26, 0.59) [0.4370]
Sleep duration	-0.3±0.2	-0.1±0.2	-0.2 (-0.67, 0.30) [0.4480]
Habitual sleep efficiency	-0.25±0.2	-0.17±0.2	-0.08 (-0.69, 0.53) [0.7898]
Sleep disturbances	-0.06±0.1	0±0.1	-0.06 (-0.35, 0.24) [0.7007]
Use of sleeping medication	0.14±0.2	0.17±0.2	-0.03 (-0.63, 0.57) [0.9155]
Daytime dysfunction	-0.14±0.1	-0.1±0.2	-0.04 (-0.48, 0.39) [0.8460]

3.4.4 BPI-Short Form

A reduction in the total pain severity score (BPI-S) was evident in both dietary interventions (Table 16). The total BPI-S scores decreased from 6.7±1.7 at baseline to 6±2.2 by the end of study in the GFD and from 6.9±1.4 at baseline to 6.3±2.1 on week 24 in the HCD group. Both dietary interventions were associated with small effect sizes in the reduction of pain severity, medium effect sizes were seen in the change of maximum pain scores on week 24 in both groups. Only time significant changes were obtained (P= 0.0003); treatment and interaction effects were non-significant. Evaluating the subcomponents scores demonstrated a time-significant reduction of the maximum pain scores, with medium effect sizes being encountered in both groups. While the effect of both dietary therapies on the minimum pain scores were negligible, average pain scores were reduced by both dietary interventions with small effect sizes. Slightly better improvement of the current pain scores was seen among patients placed on HCD as medium effect sizes were achieved (ES= 0.52) as compared to the small effect of GFD (ES= 0.42).

Non-significant differences in the interference of pain with the daily life activities were obtained. Pain interference in the HCD group decreased from 7.2±1.6 at baseline to 6.3±2.3 on week 24 as compared to a decrease from 7.1±1.7 to 6.7±2 in the GFD group. (Table 16). A medium effect size of change in the pain interference score was obtained in the HCD group by the end of study. With respect to subcomponent analysis, medium effect sizes of change in general activity and normal work were seen in the HCD group as compared to the enjoyment of life subcomponent in the GFD group.

Analysis of the mean differences of change from baseline between the two groups revealed comparable outcomes in both groups (Table 17). Non-significant differences between the two groups were obtained in both BPI-S and BPI-I scales.

Table 16. BPI total and subcomponent scores

		Baseline Mean±S.D	Week 4 Mean±S.D	Week 8 Mean±S.D	Week 12. Mean±S.D	Week 18 Mean±S.D	Week 24 Mean±S.D	
Mean BPI-severity	*GFD*	6.7±1.7	6.2±2.2	6±2	6.2±2	6±2.1	6±2.2	Interaction effect P= 0.99939
	ES		-0.29	-0.41	-0.29	-0.41	-0.41	**Time effect P= 0.0003**
	HCD	6.9±1.4	6.6±1.7	6.4±1.6	6.5±1.8	6.4±1.9	6.3±2.1	Treatment effect P= 0.4197
	ES		-0.21	-0.36	-0.28	-0.36	-0.43	
Mean BPI-interference	*GFD*	7.1±1.7	6.8±2.1	6.9±1.8	6.9±1.8	6.5±2.1	6.7±2	Interaction effect P= 0.5598
	ES		-0.18	-0.12	-0.12	-0.35	-0.23	**Time effect P= 0.003**
	HCD	7.2±1.6	6.9±1.9	6.7±1.8	6.7±1.9	6.7±2.2	6.3±2.3	Treatment effect P= 0.8958
	ES		-0.19	-0.31	-0.31	-0.31	**-0.56**	
Maximum pain	*GFD*	8.1±1.4	7.6±1.9	7.5±1.8	7.6±1.8	7.3±2	7.2±2.2	Interaction effect P= 0.9995
	ES		-0.36	-0.43	-0.36	**-0.57**	**-0.64**	**Time effect P<0.0001**
	HCD	8.1±1.2	7.6±1.6	7.5±1.7	7.5±1.8	7.3±1.9	7.3±1.9	Treatment effect P= 0.9769
	ES		-0.42	**-0.5**	**-0.5**	**-0.67**	**-0.67**	
Minimum pain	*GFD*	5.1±2.4	5.1±2.8	4.4±2.7	4.8±2.5	4.8±2.7	5±2.6	Interaction effect P= 0.9712
	ES		0	-0.29	-0.12	-0.12	-0.04	Time effect P=0.1374
	HCD	5.5±2.1	5.6±2.2	5.1±2.1	5.4±2.1	5.2±2.3	5.4±2.5	Treatment effect P= 0.2867
	ES		0.05	-0.19	-0.05	-0.14	-0.05	
Average pain	*GFD*	6.8±1.8	6±2.4	6±2.2	6.1±2.1	5.9±2.2	6±2.1	Interaction effect P= 0.6780
	ES		-0.44	-0.44	-0.39	**-0.5**	-0.44	**Time effect P= 0.0013**
	HCD	6.7±1.5	6.4±1.8	6.4±1.8	6.4±1.8	6.2±2	6.2±2.1	Treatment effect P= 0.5554
	ES		-0.2	-0.2	-0.2	-0.33	-0.33	
Current pain	*GFD*	6.7±2.1	6.2±2.7	6±2.3	6.4±2.4	6±2.4	5.8±2.5	Interaction effect P= 0.9076
	ES		-0.24	-0.33	-0.14	-0.33	-0.43	**Time effect P= 0.0009**
	HCD	7.2±1.9	6.7±2	6.5±2.1	6.5±2.1	6.6±2.5	6.2±2.6	Treatment effect P= 0.3188
	ES		-0.26	-0.37	-0.37	-0.31	**-0.53**	
General activity	*GFD*	7.5±1.9	7.2±2.3	7±2	6.9±1.9	6.5±2.3	6.8±2.4	Interaction effect P= 0.7119
	ES		-0.16	-0.26	-0.31	**-0.53**	-0.37	**Time effect P<0.0001**
	HCD	7.6±1.8	7.2±1.6	6.9±1.8	6.8±2	6.9±2.2	6.6±2.3	Treatment effect P= 0.9353
	ES		-0.22	-0.39	-0.44	-0.39	**-0.55**	
Mood	*GFD*	7.5±2.2	6.8±2.4	6.8±2.1	6.9±2	6.6±2.2	6.8±2.2	Interaction effect P= 0.9651
	ES		-0.32	-0.32	-0.27	-0.41	-0.32	**Time effect P= 0.0216**
	HCD	7.1±2.5	6.4±2.8	6.5±2.5	6.6±2.4	6.5±2.7	6.2±2.8	Treatment effect P= 0.4773
	ES		-0.28	-0.24	-0.2	-0.24	-0.36	
Normal work	*GFD*	7.3±2.3	7.2±2.3	7.1±1.9	7.2±1.9	6.8±2.3	7±2	Interaction effect P= 0.5609

	ES		-0.04	-0.09	-0.04	-0.22	-0.13	**Time effect P= 0.0153**
	HCD	7.9±1.4	7.6±1.9	7.3±1.9	7.1±2.2	7.1±2.3	6.9±2.3	Treatment effect P= 0.6248
	ES		-0.21	-0.43	**-0.57**	**-0.57**	**-0.71**	
Walking ability	*GFD*	6.9±2.4	6.5±2.5	6.6±2.1	6.6±2.1	6.2±2.3	6.3±2.3	Interaction effect P= 0.8481
	ES		-0.17	-0.12	-0.12	-0.29	-0.25	**Time effect P= 0.0350**
	HCD	7.4±1.9	7.1±1.7	7±1.8	6.7±2.1	6.9±2.3	6.6±2.3	Treatment effect P= 0.3117
	ES		-0.16	-0.21	-0.37	-0.26	-0.42	
Relation with others	*GFD*	5.8±2.9	6±2.8	6.3±2.3	6.4±2.2	5.9±2.7	6.2±2.4	Interaction effect P= 0.1633
	ES		0.07	0.17	0.21	0.03	0.14	Time effect P= 0.7374
	HCD	6.3±2.5	6.2±2.6	5.8±2.6	6.1±2.6	6±2.8	5.5±3.1	Treatment effect P= 0.7947
	ES		-0.04	-0.2	-0.08	-0.12	-0.32	
Sleep	*GFD*	7.3±2.6	6.8±2.9	7.5±2.5	6.9±2.5	6.6±2.8	6.9±2.5	Interaction effect P= 0.6453
	ES		-0.19	0.08	-0.15	-0.27	-0.15	Time effect P= 0.1029
	HCD	7.3±2.4	7.2±2.6	7.2±2.3	7±2.4	6.9±2.5	6.6±2.8	Treatment effect P= 0.9661
	ES		-0.04	-0.04	-0.12	-0.17	-0.29	
Enjoyment of life	*GFD*	7.7±2	7±2.1	7±2	7.3±2	6.8±2.2	6.7±2.2	Interaction effect P= 0.5803
	ES		-0.35	-0.35	-0.2	-0.45	**-0.5**	**Time effect P= 0.0005**
	HCD	7.2±2.3	6.6±2.5	6.4±2.4	6.2±2.3	6.5±2.5	6.1±2.6	Treatment effect P= 0.1915
	ES		-0.26	-0.35	-0.43	-0.30	-0.48	

Table 17. Mean differences of change from baseline in BPI scores

	GFD ***(Mean change±SE)***	***HCD*** ***(Mean change±SE)***	***Mean difference****: GFD-HCD* ***(95% CI)*** ***[Student's t-test P-value]***
Week 4			
BPI-severity	-0.43±0.2	-0.36±0.2	-0.07 (-0.72, 0.57) [0.8220]
BPI-interference	-0.335±0.2	-0.332±0.2	-0.003 (-0.68, 0.67) [0.9944]
Week 8			
BPI-severity	-0.7±0.2	-0.54±0.2	-0.16 (-0.83, 0.52) [0.6473]
BPI-interference	-0.2±0.3	-0.5±0.2	0.3 (-0.46, 1.06 [0.4358]
Week 12			
BPI-severity	-0.44±0.2	-0.46±0.3	0.02 (-0.66, 0.69) [0.9606]
BPI-interference	-0.2±0.2	-0.6±0.2	0.4 (-0.32, 1.07) [0.2898]
Week 18			
BPI-severity	-0.65±0.2	-0.57±0.2	-0.08 (-0.74, 0.57) [0.8044]
BPI-interference	-0.62±0.3	-0.56±0.3	-0.06 (-0.84, 0.71) [0.8676]
Week 24			
BPI-severity	-0.65±0.2	-0.66±0.3	0.01 (-0.72, 0.73) [0.9883]
BPI-interference	-0.5±0.3	-0.9±0.3	0.4 (-0.42, 1.29) [0.3119]

3.4.5 Other secondary outcome measures

Both dietary interventions were associated with minor effects on the psychological disturbances experienced by the study participants. Depressive symptoms measured with the BDI-II slightly improved in both groups with only a time-significant change (P= 0.0202) and small effect sizes (Table 18). The effects of GFD and HCD on the state and trait anxiety were almost negligible as reflected in the outcomes of the STAI scales (Table 18). The differences of change from baseline between the two groups were non-significant for BDI-II, STAI S/A and STAI T/A (Table 19).

With respect to the QOL, time-significant effect was obtained for the physical component summary of the SF-12 (P= 0.0104), whereby an improvement with a medium effect size was seen among patients in the HCD group (ES= 0.5) and a small effect size in the GFD group (ES= 0.32). Both dietary interventions had non-substantial effects on the mental component summary (Table 18). The mean differences of change from baseline between the two groups didn't show any significant difference in the QOL improvement (Table 19).

Table 18. Other secondary outcome measures

		Baseline Mean±S.D	Week 4 Mean±S.D	Week 8 Mean±S.D	Week 12. Mean±S.D	Week 18 Mean±S.D	Week 24 Mean±S.D	
BDI-II Total	*GFD*	30.5±11.1	28.8±13.5	29.2±11.1	29.5±11.4	27.7±12.5	27.3±11.7	Interaction effect P= 0.6317
	ES		-0.15	-0.12	-0.09	-0.25	-0.29	**Time effect P= 0.0202**
	HCD	29.3±11.4	27.7±11.1	26.5±12.2	26.2±12	27±13.6	26.3±13.5	Treatment effect P= 0.5134
	ES		-0.14	-0.24	-0.27	-0.20	-0.26	
BDI-II Cognitive component	*GFD*	14.7±7.3	14.2±8	14.7±6.9	14.7±6.8	14±7.5	13.4±7	Interaction effect P= 0.5537
	ES		-0.07	0	0	-0.09	-0.18	Time effect P= 0.1896
	HCD	14.1±7.6	13.2±7	12.4±7.6	12.2±7.9	12.6±8.6	12.3±8.4	Treatment effect P= 0.3572
	ES		-0.12	-0.22	-0.25	-0.19	-0.24	
BDI-II Somatic component	*GFD*	15.7±4.7	14.6±6	14.6±5.3	15±5.3	13.8±5.6	14±5.5	Interaction effect P= 0.6874
	ES		-0.23	-0.23	-0.15	-0.40	-0.36	**Time effect P= 0.0087**
	HCD	15.2±4.7	14.5±5	14.1±5.2	14±5.1	14.2±5.7	14±5.8	Treatment effect P= 0.7896
	ES		-0.15	-0.23	-0.25	-0.21	-0.25	
STAI S/A	*GFD*	36.1±11.2	35.5±11.7	36.1±10.3	37±11.7	35.2±11.3	36.6±10.8	Interaction effect P= 0.5584
	ES		-0.05	0	0.08	-0.08	0.04	Time effect P= 0.9999
	HCD	35.3±12.4	36±11.4	35.4±12.1	34.9±12.7	36.4±13.3	35.2±11.7	Treatment effect P= 0.8146
	ES		0.06	0.01	-0.03	0.09	-0.01	
STAI T/A	*GFD*	39.6±9.8	40.2±8.6	40.2±9.1	40.5±8.5	39.2±8.8	39.9±8.2	Interaction effect P= 0.4815
	ES		0.06	0.06	0.09	-0.04	0.03	Time effect P= 0.3676
	HCD	38±10.7	37.8±10.1	38.3±10.1	37.3±11.3	37.2±11.9	35.3±10.8	Treatment effect P= 0.2081
	ES		-0.02	0.03	-0.06	-0.07	-0.25	
SF-12 PCS	*GFD*	28.7±4.7	30.1±5.8	30±5.8	30±6	29.3±50.6	30.2±5.3	Interaction effect P= 0.3420
	ES		0.29	0.28	0.28	0.13	0.32	**Time effect P= 0.0104**
	HCD	27.1±5.4	27.6±4.8	29.1±5.9	28.3±5.2	29.3±6.1	29.8±5.4	Treatment effect P= 0.2607
	ES		0.09	0.37	0.22	0.41	**0.5**	
SF-12 MCS	*GFD*	31.9±9.2	30.9±10.2	32.1±9.2	32.2±10.2	32.3±10.5	33.3±10	Interaction effect P= 0.6105
	ES		-0.11	0.02	0.03	0.04	0.15	Time effect P= 0.4217
	HCD	34.4±12.2	34.5±12.1	34.1±12	37±13.2	36±13	34.6±12.6	Treatment effect P= 0.1902
	ES		0.01	-0.02	0.21	0.13	0.02	

Table 19. Mean differences of change from baseline in other secondary outcome measures

	GFD ***(Mean change±SE)***	***HCD*** ***(Mean change±SE)***	***Mean difference****: GFD-HCD* ***(95% CI)*** ***[Student's t-test P-value]***
Week 4			
BDI-II Total	-1.65±1.4	-1.57±1	-0.08 (-3.50, 3.34) [0.9619]
BDI-II Cognitive component	-0.5±0.8	-0.9±0.8	0.4 (-1.94, 2.60) [0.7714]
BDI-II Somatic component	-1.1±0.8	-0.7±0.5	-0.4 (-2.17, 1.35) [0.6399]
STAI S/A	-0.6±1.4	0.6±1.5	-1.2 (-5.28, 2.93) [0.5708]
STAI T/A	0.5±1.5	-0.1±1	0.6 (0.67, 1.75) [0.7039]
SF-12 PCS	1.4±0.9	0.5±0.9	0.9 (-1.74, 3.50) [0.5045]
SF-12 MCS	-1±1.7	0.05±1.5	-1.05 (-5.49, 3.48) [0.6562]
Week 8			
BDI-II Total	-1.2±1.3	-2.8±1.5	1.6 (-2.47, 5.55) [0.4449]
BDI-II Cognitive component	-0.06±0.8	-1.7±1	1.64 (-0.93, 4.22) [0.2075]
BDI-II Somatic component	-1.08±0.7	-1.1±0.7	0.02 (-2.03, 2.06) [0.9889]
STAI S/A	0.06±1.6	0.02±1.5	0.04 (-4.33, 4.39) [0.9883]
STAI T/A	0.5±1.5	0.3±1.2	0.2 (0.16, 1.94) [0.9327]
SF-12 PCS	1.4±0.8	2±1	-0.6 (-3.28, 2.11) [0.6656]
SF-12 MCS	0.2±1.4	-0.4±1.8	0.6 (-4.06, 5.22) [0.8038]
Week 12			
BDI-II Total	-0.9±1.3	-3±1.7	2.1 (-2.26, 6.45) [0.3389]
BDI-II Cognitive component	-0.06±0.9	-1.9±1.2	1.84 (-1.23, 4.92) [0.2359]
BDI-II Somatic component	-0.8±0.6	-1.1±0.7	0.3 (-1.55, 2.31) [0.6962]

	GFD ***(Mean change±SE)***	***HCD*** ***(Mean change±SE)***	***Mean difference****: GFD-HCD* ***(95% CI)*** ***[Student's t-test P-value]***
STAI S/A	0.9±1.8	-0.5±1.7	1.4 (-3.61, 6.34) [0.5869]
STAI T/A	0.85±1.5	-0.65±1.4	1.5 (-2.50, 5.52) [0.4557]
SF-12 PCS	1.3±0.8	1.1±0.9	0.2 (-2.32, 2.62) [0.9029]
SF-12 MCS	3.6±2	2.5±2.1	1.1 (-4.66, 6.86) [0.7039]
Week 18			
BDI-II Total	-2.7±1.3	-2.3±1.6	-0.4 (-4.73, 3.95) [0.8572]
BDI-II Cognitive component	-0.7±1	-1.4±1.1	0.7 (-2.30, 3.77) [0.6305]
BDI-II Somatic component	-1.9±0.6	-1±0.7	-0.9 (-2.89, 1.01) [0.3397]
STAI S/A	-0.9±1.7	1.1±1.5	-2 (-6.51, 2.59) [0.3929]
STAI T/A	-0.4±1.5	-0.7±1.3	0.3 (-3.75, 4.24) [0.9024]
SF-12 PCS	0.7±0.8	2.2±1.1	-1.5 (-4.28, 1.28) [0.2839]
SF-12 MCS	0.4±1.4	1.6±1.9	-1.2 (-5.99, 3.67) [0.6331]
Week 24			
BDI-II Total	-3.1±1.2	-2.9±1.7	-0.2 (-4.45, 4.06) [0.9282]
BDI-II Cognitive component	-1.3±0.8	-1.8±1.1	0.5 (-2.39, 3.25) [0.7606]
BDI-II Somatic component	-1.7±0.6	-1.2±0.8	-0.5 (-2.53, 1.51) [0.6150]
STAI S/A	0.6±1.8	-0.2±1.6	0.8 (-4.06, 5.55) [0.7574]
STAI T/A	0.2±1.6	-2.6±1.5	2.8 (-1.54, 7.19) [0.2005]
SF-12 PCS	1.5±0.8	2.6±1	-1.1 (-3.72, 1.56) [0.4179]
SF-12 MCS	1.5±1.4	0.1±1.6	1.4 (-3.07, 5.69) [0.5512]

3.4.6 Percentage of responders as evaluated by primary and secondary outcome measures

As it is shown in Table 20, the proportions of responders, as evaluated by the reduction in the gluten sensitivity symptoms count, were comparable in both groups: 46% of patients in the GFD group had at least 20% reduction in the symptoms count as compared to 50% in the HCD group. Comparable proportions of patients achieved at least 30% reduction in the total number of patients in both groups (31.4% in GFD versus 35% in HCD group). Fifty % reduction in the total symptoms count was achieved by only 11.4% and 10% of patients in GFD and HCD groups, respectively.

Considerable proportion of patients had at least a 20% reduction in the FIQR scores in the GFD group (40%) and in the HCD group (32.5%). The percentage of patients decreased to 22.8% in the GFD group and 27.5% in the HCD group, when a 30% reduction in the total FIQR score was considered. Only 5 patients in the HCD group and none in the GFD group recorded a drop of more than 50% in the total FIQR score.

In general, the percentages of responders in the remaining outcome measures were slightly higher in the HCD group as compared to the GFD group. Contingency analysis showed the absence of any significant difference in the proportion of responders of both groups in all of the outcome measures evaluated.

Table 20. Percentage of responders as evaluated by secondary outcome measures

	GFD (%)	**HCD (%)**	**P-value**
Gluten sensitivity symptoms count			
≥20% reduction	46	50	0.8178
≥30% reduction	31.4	35	0.8091
≥50% reduction	11.4	10	1
FIQR			
≥14% reduction	54	37.5	0.1687
≥20% reduction	40	32.5	0.6304
≥30% reduction	22.8	27.5	0.7913
≥50% reduction	0	12.5	0.0569
PSQI			
≥20% reduction	23	32.5	0.4426
≥30% reduction	14	17.5	0.7615

≥50% reduction	0	12.5	0.0569
BPI-S			
≥20% reduction	28.5	30	1
≥30% reduction	17	22.5	0.7732
≥50% reduction	5.7	7.5	1
BPI-I			
≥20% reduction	28.5	35	0.6224
≥30% reduction	14	22.5	0.3926
≥50% reduction	5.7	12.5	0.4380
BDI-II			
≥20% reduction	20	25	0.4125
≥30% reduction	31.4	40	0.7831
STAI S/A			
≥20% reduction	20	15	0.7610
≥30% reduction	11.4	12.5	1
STAI T/A			
≥20% reduction	8.6	22.5	0.1240
≥30% reduction	2.8	15	0.1132

3.4.7 PGI-S and PGI-I

Patients in the GFD group who evaluated the severity of their illness as normal or borderline ill increased from 1 at baseline to 2 by the end of the study. Similar number of patients in the HCD group (n= 2) classified themselves as normal or borderline ill by the end of the study (Figures 8 and 9). The number of patients perceiving their illness as mild increased from 1 at baseline to 3 on week 24 in the GFD group and from 1 to 2 in the HCD group. While the number of moderately ill patients increased from 7 to 10 in the GFD group, their number decreased from 8 to 7 in the HCD group. The additional 3 patients with “moderately ill” classification in the GFD group had initially classified their illness as markedly ill. An interesting observation was the change achieved in the number of patients placed on a GFD who classified their illness as markedly ill at baseline; their number decreased from 13 to 7 on week 24. The 6 patients, who initially classified their illness as markedly ill, provided a better evaluation of the severity of their illness by the end of the study: 1 patient had a normal classification, 2 mildly ill, and 3 moderately ill. On the other hand, the number of patients who classified their illness as markedly ill in the HCD increased from 11 at baseline to 13 on week 24. The number of

patients perceiving their illness as severely was not changed in the GFD group, whereas a decrease from 9 to 5 patients was achieved in the HCD group.

With respect to PGI-I, after completing 24 weeks of dietary therapy, similar percentages of patients who had a post-treatment perception as being “much better” were observed in both groups (22.8% in GFD and 20% in HCD) (Figure 10). The percentage of patients who were “slightly better” was higher among the HCD group (30%) than in GFD group (20%). The percentage of patients experiencing “no change” was 22.8% in the GFD group as compared to 17.5% in the HCD group. Five% of patients in the HCD group had a “worse” impression as compared to 3% in the GFD group.

Figure 8. Change of PGI-S between baseline and endpoint for completers in GFD group

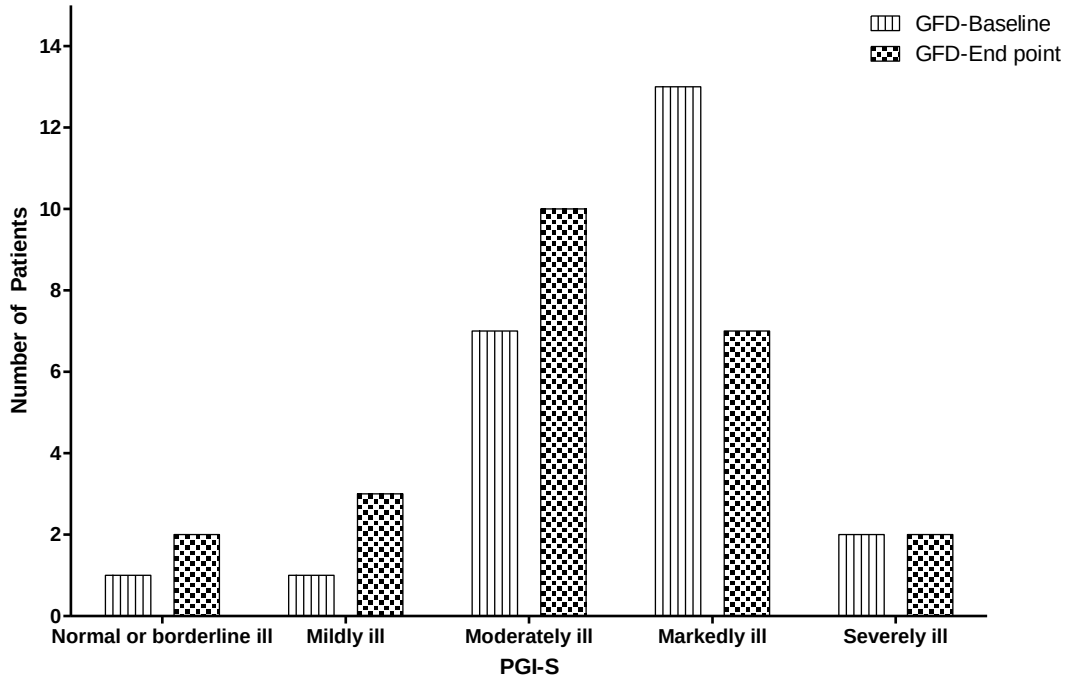


Figure 9. Change of PGI-S between baseline and endpoint for completers in HCD group

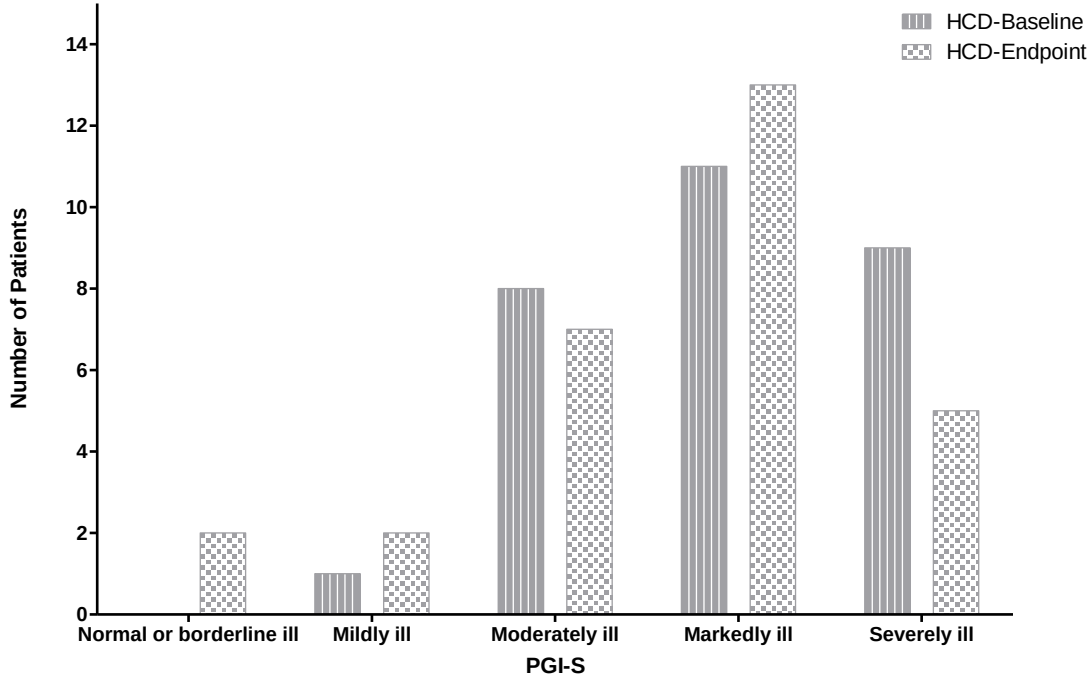
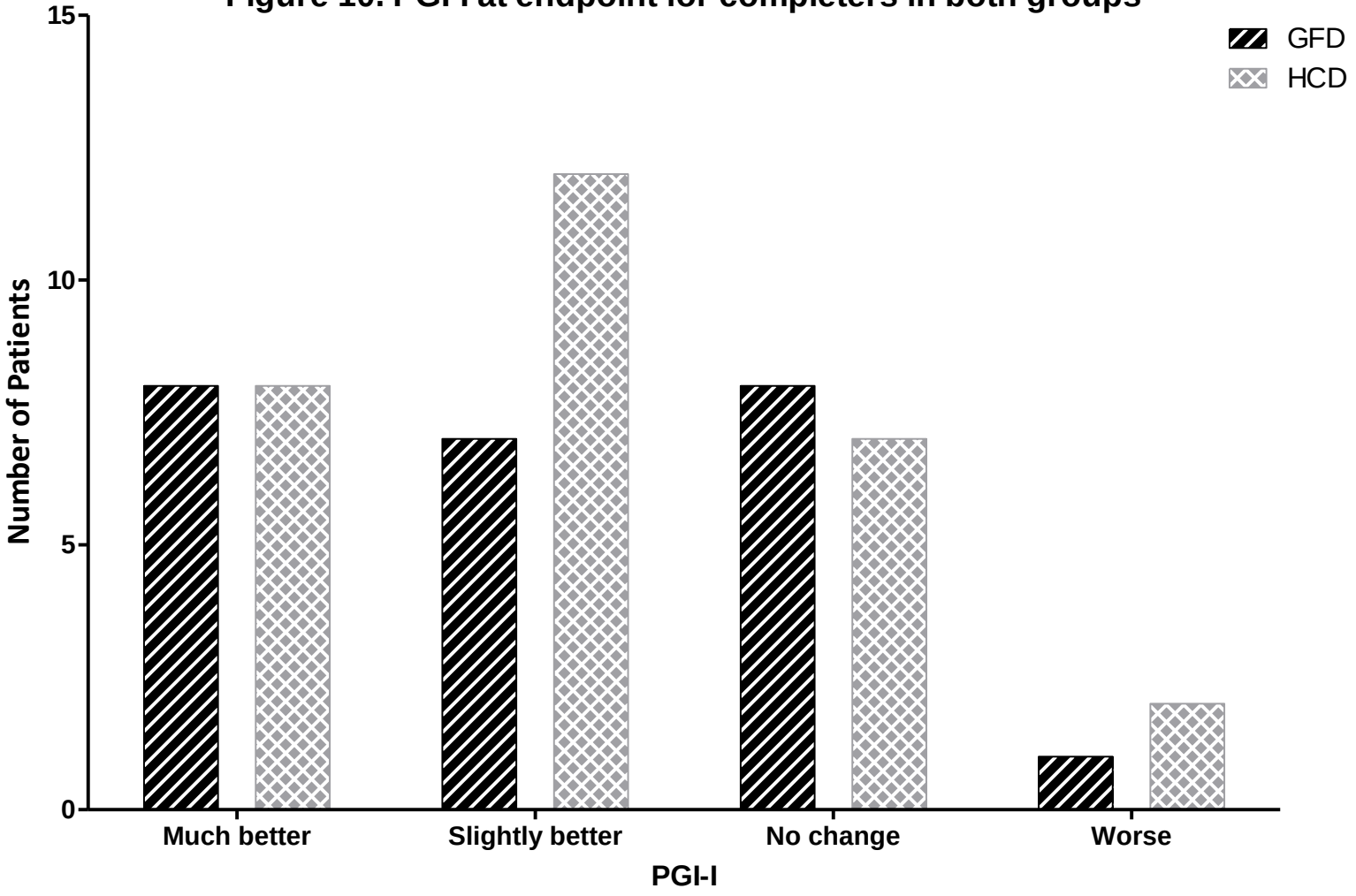


Figure 10. PGI-I at endpoint for completers in both groups



3.4.8 Safety of dietary interventions

Both dietary interventions were well tolerated with only mild and transient adverse events being reported by some patients as illustrated in Table 21. No dropouts due to adverse events were encountered in any of the two groups.

At week 12, both dietary interventions were not associated with any abnormal alteration in the laboratory parameters; neither in the complete blood counts nor in the biochemistry profile of patients.

Table 21. Adverse events

	GFD N (%)	HCD N (%)
Number of patients who reported AE	2 (5.7)	6 (15)
Hand tremor	1 (2.8)	0
Gingivitis	1 (2.8)	0
Nausea/Vomiting	0	2 (5)
Headache	0	2 (5)
Flu-like symptoms	0	1 (2.5)
Sole feet pain	0	1 (2.5)
Diarrhea	0	1 (2.5)

3.4.9 Post-hoc analysis

In order to control for the basal BMI difference between the two groups, post-hoc analysis comparing the changes in the primary and secondary outcome measures between the corresponding weight groups in the two groups was carried out whereby patients were categorized into normal, overweight or obese based on their basal BMI values. Results demonstrated non-significant differences in the change of all outcome measures, except BMI, when comparing the effect of GFD and HCD on patients of corresponding weight groups (Tables 22 and 23). Changes in the BMI revealed a more significant drop among obese patients; statistical significance was achieved only in the GFD group, whereby the change increased linearly in function of the basal BMI scores.

The assessment of the influence of basal BMI on the response to both GFD and HCD was conducted by considering the whole sample. Then, we compared the change in all outcome measures between patients of different weight groups. Only BMI drop was significantly greater among obese patients as compared to other weight groups, whereas all the remaining outcome measures were associated with non-significant changes.

Correlation analysis of the basal anthropometric measures (BMI, waist circumference, waist-to-height) with the baseline score of gluten sensitivity symptoms and its subcomponents scales didn’t show the presence of any significant correlation.

Table 22. Mean change from baseline among the different weight groups of patients placed on GFD

	Normal N= 14	Overweight N= 11	Obese N= 10	One-way ANOVA
BMI (Kg/m^2)	-0.14±1.1	-1.17±1*	-1.2±0.8*	P= 0.0134
Gluten sensitivity symptoms	-2.3±2.2	-3±2.9	-2.1±2	P= 0.6577
FIQR	-11.9±12.1	-5.9±10.9	-8.8±12.9	P= 0.4631
PSQI	-1.6±2.3	-1.2±4.3	-0.2±2.9	P= 0.5797
BPI-S	-0.8±1.1	-0.3±1.5	-0.8±1.8	P= 0.6537
BPI-I	-0.98±2.3	-0.2±1.1	0.02±1.2	P= 0.3285
BDI-II	-6.1±7.4	-1.6±5.3	-0.6±6.6	P= 0.0993
STAI S/A	0.42±11.8	-0.4±11.6	1.8±8.4	P= 0.9
STAI T/A	-1.2±12.5	-0.2±7	2.7±5.7	P= 0.5993
SF12 PCS	2±5.3	1±4.5	1.5±4.3	P= 0.8863
SF12 MCS	2.9±10.1	1.7±7.5	-0.8±6	P= 0.5505

*: P<0.05 compared to normal IMC patients using Tukey's Multiple Comparison Test. Results are presented as mean change±S.E

Table 23. Mean change from baseline among the different weight groups of patients placed on HCD

	Normal N= 7	Overweight N= 14	Obese N= 19	One-way ANOVA
BMI (Kg/m^2)	-1±0.8	-0.9±1.3	-1.5±1.5	P= 0.4986
Gluten sensitivity symptoms	-1.4±2	-2.3±2.6	-2.2±2.5	P= 0.7321
FIQR	-10.8±28	-8.3±20.2	-8.1±17.3	P= 0.9545
PSQI	-3.4±5.5	-0.3±4.4	-0.3±4.3	P= 0.2674
BPI-S	-0.4±2	-1±1.6	-0.5±1.6	P= 0.6259
BPI-I	-0.8±2.5	-1±1.98	-0.8±1.8	P= 0.9586
BDI-II	-4.4±12.6	-2.7±9.4	-2.6±11.7	P= 0.9272
STAI A/E	0.7±16.2	-1.9±7.3	0.8±9.8	P= 0.7391
STAI A/R	5.3±8.9	-4±5.5	-4.5±11	P= 0.0532
SF12 PCS	0.7±6.3	1.7±6.6	3.97±6.5	P= 0.4469
SF12 MCS	1.8±13.6	2.7±7.7	-2.4±10.9	P= 0.3527

Results are presented as mean change±S.E

4. Discussion

4.1 Overview of the outcomes

The results of our study demonstrated the absence of any significant differences between gluten-free and hypocaloric diets with respect to the change in the number of experienced gluten sensitivity symptoms (gastrointestinal and extraintestinal), fibromyalgia symptoms (including the impact and the severity of the disease), sleep problems, pain intensity, depression, anxiety, and quality of life when adopted by patients with fibromyalgia experiencing overlapping gluten sensitivity symptoms. Thus, these outcomes do not support the hypothesized role for gluten sensitivity in the underlying pathophysiology of the gastrointestinal and extraintestinal manifestations in fibromyalgia.

4.2 Effect of the dietary interventions on the primary outcome measure (Gluten Sensitivity Symptoms)

Our results did not show any significant difference between the two dietary interventions, GFD and HCD, on the change of the total number of gluten sensitivity symptoms with only a time significant change being noted. Thus, despite its specificity, GFD was not superior to HCD in reducing these manifestations.

To our knowledge, our primary outcome measure which aims at evaluating the change in the total number of gluten sensitivity symptoms has not been evaluated in any previous study. Thus, it is not possible to compare these outcomes with the previously published data in the literature.

Gastrointestinal symptoms, whether IBS-like or non-specific, are commonly reported by patients with fibromyalgia (Slim et al. 2015). In addition, as reported in several studies, food allergies and intolerances are frequently seen among this group of patients (Arranz et al. 2012, Berstad et al. 2012, Puccio et al. 2013). These observations, along with the evidence of a clinically significant overlap in the symptomatologic spectrum between fibromyalgia and gluten-related disorders (Garcia-Leiva et al. 2015), led to a suspicion of an underlying role of gluten sensitivity in provoking part of the manifestations

experienced by patients with fibromyalgia. However, the outcomes of our study do not support a specific role of gluten sensitivity which doesn't seem to be the underlying factor behind the appearance of these manifestations, supporting the notion of the presence of broader factors contributing to the appearance of this complex symptomatologic spectrum such as hypersensitivity to FODMAPs among other possible pathophysiologic mechanisms.

FODMAPs are short-chain carbohydrates poorly absorbed in the small intestine. They include fructans, galactose, lactose, fructose and sugar alcohols that are found in a wide variety of dietary sources such as certain fruits (apple, pear, peach, watermelon, etc.), cereals (wheat, rye and barley), milk, and yogurt, among others (Biesiekierski et al. 2013). The fructans oligosaccharides are the specific carbohydrates present in wheat whose various constituents have been linked to distinct pathologic effects (Mansueto et al. 2014). FODMAPs have been proposed to play a role in the pathophysiologic mechanisms underlying NCGS. It has been recently postulated that the triggers of NCGS symptoms are not limited to the gliadin, non-gliadin parts of gluten or gluten contaminants but rather they might include other wheat components such as amylase-trypsin inhibitors or fermentable oligo-, di-, mono-saccaharides and polyols (FODMAPs) (Mansueto et al. 2014). Their dietary reduction in patients with IBS and NCGS was linked to a significant symptomatic relief as reported in the placebo-controlled, cross-over study conducted by Biesiekierski et al. (Biesiekierski et al. 2013).

Sensitivity to FODMAPs might constitute the common base for the sensitivity to various food components, as this broad family includes the sensitivity to lactose and wheat (gluten). To our knowledge, the specific role of FODMAPs in fibromyalgia and the possible underlying mechanisms associated to its possible effects in fibromyalgia are not yet investigated. The current experience with FODMAPs restriction diet in IBS has revealed promising outcomes (de Roest et al. 2013, Halmos et al. 2014), as it has been linked to improved IBS symptomatology of pain, bloating, flatulence and nausea in addition to improved quality of life (Staudacher et al. 2014). These outcomes encourage undertaking the adequate investigations to explore any possible role of these nutritional constituents in fibromyalgia.

In a recent study published by Rodrigo et al. (2014), the effect of GFD diet was evaluated in two groups of patients with fibromyalgia; one group consisted of patients with fibromyalgia who were also diagnosed with comorbid IBS and lymphocytic enteritis (LE) and another group of patients with fibromyalgia who were only diagnosed with comorbid IBS in the absence of LE. Interestingly, after one year of GFD adoption, the former group demonstrated partial (26 to 30%) but statistically significant improvement in the majority of the outcome measures being evaluated (FIQ, Pain VAS, TP count, gastrointestinal symptoms VAS and fatigue), whereas the latter group experienced negligible improvements. This study constitutes another evidence for the absence of a specific beneficial effect of GFD in the general population of patients with fibromyalgia, emphasizing the idea that the potential benefit of such dietary intervention could be limited to a subgroup of patients with fibromyalgia whom in the study of Rodrigo et al. (2014) were defined as those presenting comorbid IBS and LE. This study also serves in confirming the relatively elevated prevalence of LE among patients with FMS and IBS, whereby 58 subjects out of the 229 recruited patients tested positive for intraepithelial lymphocytes (IEL) (>25 IEL/100 enterocytes) (Rodrigo et al. 2014). Thus, this signals toward an underlying immune reaction, although mild, that is taking place in this subgroup of patients with fibromyalgia. This reaction could be provoked by NCGS, as postulated by the authors of the aforementioned study, or by other forms of sensitivity reactions.

Evaluating the effect of weight loss in fibromyalgia has been undertaken in two previous clinical studies; one case series (Shapiro et al. 2005) and another randomized, single-blinded, parallel trial (Senna et al. 2012). Both studies concluded beneficial effects of weight loss in fibromyalgia, recommending its endorsement as part of the multidisciplinary treatment of fibromyalgia. Thus, it is possible that our selection of an active comparator, whose benefit has been already demonstrated, might be behind the lack of intergroup variation between the two dietary interventions. Our study would have been probably associated with different outcomes (possibly statistically significant), had a placebo arm been included. However, designing a placebo arm in dietary studies is difficult (Bennett 2002), especially when evaluating the efficacy of elimination diets, as it is important in this case to ensure that the quantity and types of nutritional intake are

closely matched as much as possible (Yao et al. 2013). Additionally, it is important to highlight that dietary interventions whose objective is to vary the intake of a particular nutritional ingredient will usually lead to consequential changes in the intake of other nutrients (Webb 2012).

Another aspect that influenced our choice of adopting an active comparator group was related to ethical issues, as the use of an active comparator ensured respecting the ethical standards in clinical investigation represented in the patients' right to access adequate medical care, given the chronic nature of fibromyalgia and the long duration of the study.

4.3 Effects on secondary outcome measures

4.3.1 Anthropometric measures

As expected, a more significant drop in the BMI and waist circumference achieved by patients in the HCD group as compared to those placed on a GFD. It is noteworthy the drop in the BMI among patients in the GFD group. While there is no scientific evidence supporting weight control using GFD, it is known that adherence to GFD is linked to a dietary pattern that limits the intake of certain foods such as lower intake of carbohydrates, fibers and certain minerals (Marcason 2011). On the other hand, it has been reported that there is a trend in replacing the gluten-derived carbohydrates in GFD with increased consumption of fats, proteins and hypercaloric beverages (Valletta et al. 2010), which explains the hypercaloric content of GFD and the subsequent weight gain reported in several studies (Dickey and Kearney 2006, Valletta et al. 2010). These opposing points of views of the effect of GFD on the BMI were reflected in the study conducted by Cheng et al., whereby 66% of celiac disease patients who were underweight gained weight after the long-term adoption of GFD whereas 54% of the overweight and 47% of obese patients lost weight (Cheng et al. 2010).

In our study, 60% of patients who were placed on GFD were classified as overweight or obese. In order to identify the attribution of each subgroup on the BMI changes, we analyzed the change in the BMI between baseline and endpoint for normal BMI patients

and for those who were classified as overweight or obese and it turned out the drop of the BMI in the first group was -0.14 as compared to -1.17 and -1.3 in the latter groups, respectively .These outcomes were consistent with the findings reported by Cheng et al. (2010).

It is important to highlight that the underlying mechanism behind weight gain upon the adoption of GFD has been only studied in celiac disease patients who are characterized by abnormal intestinal absorption; thus, the adoption of GFD is capable of restoring the adequate absorption and subsequently leads to weight gain (Kabbani et al. 2012). On the other hand, it has been suggested that gastrointestinal manifestations in fibromyalgia could be attributed to increased intestinal permeability. This hypothesis has been confirmed exclusively in an exploratory study including 45 patients with fibromyalgia who manifested increased intestinal permeability as compared to healthy volunteers (Goebel et al. 2008). In our study, patients assigned to GFD intervention were informed of the absence of any caloric restriction for their diet and elimination of gluten, in its various forms, is the only governing regulation. Thus, the drop in the average BMI was an interesting and unexpected observation.

Previous studies, conducted by Rodrigo et al. (Rodrigo et al. 2013, Rodrigo et al. 2014), assessing the role of GFD in patients with fibromyalgia and celiac disease and in patients with fibromyalgia, IBS and LE didn't evaluate the anthropometric measures. On the other hand, these measures were evaluated in the two clinical studies assessing the effect of weight loss on the general symptomatology of fibromyalgia (Shapiro et al. 2005, Senna et al. 2012). In the study conducted by Shapiro et al. (2005), the 20-week dietary and physical activity program in fibromyalgia was associated with 1.6 Kg/m^2 drop in the BMI scores (ES: 0.26) as compared to 3.27 Kg/m^2 drop (ES: 2.33) in the 24-week weight loss program among patients with fibromyalgia conducted by Senna et al. (2012). The BMI drop (ES: 0.23) in the HCD arm of our study was slightly lower than what was obtained by Shapiro et al. (2005) and much lower than the BMI drop seen in the study conducted by Senna et al. (2012).

The differences in the BMI changes between our study and the other two studies evaluating HCD in fibromyalgia (Shapiro et al. 2005, Senna et al. 2012), could be

attributed to the degree of strictness of the caloric restriction and to the baseline differences in the BMI scores. With respect to the dietary restriction program, the first study conducted by Shapiro et al. was limited to 1200-1500 Kcal/day which was equivalent to the dietary program adopted in our protocol; on the other hand, the study conducted by Senna et al. imposed a more stringent dietary program limited to a maximum daily intake of 1200 Kcal/day. It is also important to highlight the combination therapy program adopted by Shapiro et al., whereby dietary restriction program was combined with physical activity (30 min of moderate intensity physical activity per day) and stimulus control (consisting of various contingency management techniques for weight control). Moreover, one of the the key selection criteria in the study conducted by Shapiro et al. was a baseline BMI score > 25 Kg/m^2, thus excluding patients with fibromyalgia of normal weight which led to the inclusion of patients with significantly higher baseline BMI. In fact, the baseline BMI scores of the patients included in the studies of Senna et al. and Shapiro et al. were 32.3±1.4 and 35.4±6.1 Kg/m^2, respectively. It can be noted the remarkably higher baseline BMI scores when compared to our study mean baseline BMI score (30.2±5.3). With respect to waist circumference changes, while Senna et al. didn't report the changes in waist circumference of their study sample, the changes in the waist circumference in our study (-4.6±1.1 centimeters, ES: 0.5) were comparable to that observed in the study conducted by Shapiro et al. (-2±1.6 inches, ES: 0.4)

Three other studies evaluating the effect of dietary interventions in fibromyalgia reported anthropometric data outcomes. In the first controlled non-randomized study, the adoption of Vegan diet by 18 patients with fibromyalgia over a period of 12 weeks was associated with a more significant drop in the BMI as compared to 15 patients placed on an omnivorous diet (BMI reduction values were not reported) (Kaartinen et al. 2000). In the second study also of quasi-experimental design, Michalsen et al. (2005) reported a weight drop of 1.2 Kg among 21 patients with fibromyalgia who were placed on a Mediterranean diet as compared to 3 Kg drop among 30 patients who adopted fasting therapy (corresponding BMI values were not provided).

Another study that seems worthy to mention, is the one published by Saber et al. (2008) that examined the effects of bariatric surgery in 10 patients suffering fibromyalgia and morbid obesity that found a significant drop in the BMI (14.5 Kg/m^2 drop) among these patients. Although this kind of intervention cannot be considered as a dietary measure, it is interesting because it was associated to pain improvement as it is detailed below.

4.3.2 Fibromyalgia symptomatology (FIQR)

Both dietary interventions led to an improvement in the overall symptomatology of fibromyalgia as demonstrated by the drop in the total FIQR scores, whereby it was reduced by an average of 9.2±2 points with GFD and 8.7±3.2 points with HCD and medium effect sizes being achieved by both interventions at the end of the study. In fibromyalgia, minimally clinically important difference derived from FIQ changes was reported at a cut-off point of 14% from baseline based on which patients were classified as responders (Bennett et al. 2009). Although the newer version of the FIQR includes modified questions and additional ones related to memory, tenderness, balance and environmental sensitivity, it is important to mention that the validation of the newer FIQR revealed comparable scoring characteristics to the original FIQ and thus allowing the direct comparison between FIQR outcomes and those of the FIQ (Bennett et al. 2009). Hence, although not completely accurate, extrapolation of the cut-off point (14% reduction) defining the response can be possibly applied on the FIQR as well; 54% of our patient sample placed on the GFD were capable of achieving this drop as compared to 37.5% in the HCD group.

In the study conducted by Rodrigo et al. (2013), GFD adoption by patients with comorbid FMS/IBS/CD led to a -37.7 (ES: 13) mean difference of change from baseline in the FIQ score. Given the established prevalence of extraintestinal manifestations in celiac disease (Cranney et al. 2003, Zipser et al. 2003, Hernandez and Green 2006) and their similitude with fibromyalgia symptoms (Garcia-Leiva et al. 2015), the improvement of fibromyalgia-like manifestations upon the adoption of GFD in this population could be part of the positive prognosis of this dietary intervention on the

celiac disease process. However, these outcomes are not capable of providing a definitive answer about the specificity of this dietary intervention in the improvement of fibromyalgia symptoms in non-celiac patients.

In the second study of this group (Rodrigo et al. 2014), the adoption of GFD by patients diagnosed with FMS/IBS/LE led to a significant drop in the FIQ score (20-point drop); however, in patients without LE the drop in the FIQ only reached 2.3 points. It was not possible to calculate effect sizes since the SDs were no provided in this publication.

With respect to the two previous studies evaluating the impact of weight loss on the overall symptomatology in fibromyalgia, both of them were associated with comparable outcomes of moderate effect sizes. In the study of Shapiro et al. (2005), the total FIQ was reduced by -10.5±18.3 (ES= 0.7) and a closer value of FIQ mean change from baseline was seen in the study of Senna et al. (2012) with a mean change of -7.6 (ES: 0.6). Both of these studies (Shapiro et al. 2005, Senna et al. 2012) recruited patients with moderate disease severity as reflected in the baseline FIQ scores (56.7±14 in the former and 54.6±13.1) as compared to more severe clinical profile of the subjects recruited to the HCD arm of our study (70.4±16.1).

The impact of other types of dietary interventions on improving the overall symptomatology in fibromyalgia has been evaluated in three studies:

In the case-series study conducted by Donaldson et al. (2001), the FIQ scores decreased by 23.8 (ES: 1.7) following 28 weeks of raw vegetarian diet adoption by 30 patients with fibromyalgia. In another case-series study of 8 patients with fibromyalgia, a modified elimination diet and a novel phytonutrient-rich medical food was used to increase the excretion of toxic elements (Lamb et al. 2011); it led to a 10-point drop in the FIQ scores after 4 weeks of using this dietary intervention. In the quasi-experimental study conducted by Michalsen et al. (2013), the use of conventional rheumatologic therapy by 21 patients with fibromyalgia (physiotherapy, hydrotherapy, thermal therapy, psychosomatic therapy, aerobic exercise, pool exercise, cognitive behavioural therapy and education) led to a 4.1 points (ES: 0.46) decrease in the total FIQ score which was non-significantly different from the change of -6.6 (ES: 0.44) achieved by the integrative

treatment approach followed by 30 patients with fibromyalgia (conventional therapy in additional to fasting therapy included 1–2 days of consuming 800 kcal/day, consisting of fruit, rice or potatoes according to patient choice, followed by 7–8 days of caloric restriction to <500 kcal/day).

The effect of the majority of the so-far evaluated dietary interventions in fibromyalgia, despite the variability in their type of nutritional intervention, achieved a partial improvement in the overall symptomatology of fibromyalgia as reflected by the changes in the FIQ and FIQR scores. However, with the exception of the two randomized clinical trials of Azad et al. (2000) and Senna et al. (2012), the levels of evidence provided by the studies assessing nutritional interventions are relatively low as they are characterized by small sample sizes and they are either non-controlled or controlled but non-randomized.

4.3.3 Sleep disturbances (PSQI)

Both dietary interventions were associated with small effect sizes on sleep improvement as reflected in the change of PSQI total scores. However, it was notable the strong effect size achieved by GFD in improving the subjective sleep quality subcomponent on week 18 (ES: 0.83) which turned into medium size on week 24 (ES: 0.67). Unfortunately, sleep disturbances were not evaluated in the two studies of Rodrigo et al. (2013, 2014). Studies assessing the benefits of GFD in improving sleep disturbances are scarce. In a cross-sectional study evaluating sleep disturbances in celiac disease, it was concluded that patients with celiac disease experienced higher sleep disturbances than healthy volunteers and that GFD was not associated with any improvement in the PSQI score (Zingone et al. 2010). It is important to highlight that although celiac disease patients in this study recorded pathologic scores of PSQI (>5), it was much lower than the average score of PSQI observed in patients with fibromyalgia (patients with celiac disease at time of diagnosis: 6.2±3.64; patients with celiac disease placed on GFD over at least 1 year: 5.2±2.6; patients with fibromyalgia in our total study sample: 15±3.9).

With respect to the HCD, in the study conducted by Senna et al. (2012), the use of HCD led to a 2-point drop in the PSQI score (ES: 0.68) as compared to a 0.8-point decrease in the total PSQI score achieved by the end of our study in the HCD arm (ES: 0.44). Patients in the study of Senna et al. (2012) had lower sleep disturbances at baseline as compared to patients recruited into the HCD arm in our study (6±2.9 vs 14.4±3.8). The beneficial role of weight loss in improving sleep quality has been mainly linked to the positive impact of weight loss on obstructive sleep apnea, which is commonly seen in obese subjects. This beneficial effect has been well established, as a 10% reduction in body weight predicts an improvement of 26-32% in the apnea-hypopnea index (Peppard et al. 2000).

In fibromyalgia, sleep problems originate mainly from non-restorative and non-refreshing sleep which is reported by more than 90% of patients (Moldofsky 2008). Disrupted sleep patterns in fibromyalgia include: phasic α EEG sleep, frequent cyclic alternating EEG sleep pattern and shorter stage-2 duration preceding the slow-wave sleep (Spaeth et al. 2011). In a recently published study, sleep apnea was demonstrated to be prevalent among patients with fibromyalgia accounting for 45% of the study sample (n= 133), twice as common in fibromyalgia as compared to the general population (Rosenfeld et al. 2015). Thus, these figures advocate a potential benefit of weight loss programs on sleep disturbances in fibromyalgia. Reasons behind the minor effect on sleep in our study sample receiving HCD could be attributed to several factors which include the modest weight loss (3 Kg decrease, 4% decrease in body weight within the HCD group) achieved in our study, the borderline obese classification of the patients recruited into our study sample receiving HCD (mean BMI at baseline: 30.2±5.3) and the difficult-to-predict compliance rates with the dietary intervention.

The effects of other dietary interventions on sleep disturbances in fibromyalgia were evaluated in 3 clinical studies; one randomized (Azad et al. 2000) and 2 quasi-experimental studies (Kaartinen et al. 2000, Michalsen et al. 2013). In the first study, in which patients were randomized to either a vegetarian diet or amitriptyline therapy, the percentage of patients with sleep disturbances increased in the former group as

compared to a complete resolution in the amitriptyline group (Azad et al. 2000). The dichotomous evaluation of sleep disturbances in this study limits the capability of quantifying and comparing the approximate effect of these interventions on sleep disturbances. On the other hand, the vegan diet adopted by 18 patients recruited to the study conducted by Kaartinen et al. (2000) led to a more significant improvement as compared to those who were placed on an omnivorous diet (n= 15); however, results were reported in figures and it was not possible to retrieve data for comparison. In the study of Michalsen et al. (2013), the integrative therapy was significantly better than conventional therapy in improving sleep disturbances (evaluated with VAS 0-100) with a mean difference of change reaching -16.5 (95% CI -30, -3.1); however, in-line with our outcomes, a small effect size was recorded (ES: 0.45).

4.3.4 Pain

Both dietary interventions evaluated in our study had small effect sizes on the improvement of BPI-severity. However, HCD was associated with a better improvement in BPI-I with a medium effect size. In the study conducted by Rodrigo et al. (2014), pain was evaluated using an 11-point VAS on which GFD led to 2.4-point drop (ES could not be calculated since SDs are not provided). On the other hand, lower effects were seen in our study as GFD was associated with a 0.65-point drop in BPI-S and 0.5-point drop in BPI-I. While the scientific literature is rich in material investigating the role of GFD in reducing GI-related pain and other celiac symptoms, we did not find any evidence for a potential role of GFD in reducing generalized body or chronic pain.

In the literature, several cross-sectional studies demonstrated the existence of a significant correlation between obesity and chronic pain conditions such as fibromyalgia, rheumatoid arthritis, osteoarthritis and low back pain (Arranz et al. 2014). Obesity in fibromyalgia has been shown to be significantly correlated to increased pain sensitivity (Arranz et al. 2014). However, prospective studies evaluating the role of weight loss in improving pain in fibromyalgia are scarce.

Patients evaluated in the study conducted by Shapiro et al. (2005) demonstrated relatively comparable outcomes at the level of pain scores reduction; the multidimensional pain inventory evaluating pain severity decreased by 0.5 points (ES: 0.44) and large effect sizes were achieved in the change of pain interference scores (ES: 0.83). In the study conducted by Senna et al. (2012), pain was evaluated using a subitem of the FIQ scale and it was not possible to compare the results due to limited data provided (the change in the pain score was not reported); however, the authors claimed that pain scores were significantly better among those who lost weight as compared to the controls who didn't.

The retrospective case-series of Saber et al. (2008) previously mentioned found a decrease in the median VAS pain scores from 9 to 3 points that was accompanied by a substantial reduction in analgesics intake by 8 of the 10 evaluated patients.

Several underlying pathophysiologic mechanisms have been proposed to explain the correlation between obesity and chronic pain. In obese subjects, the role of the proinflammatory state, through elevated levels of inflammatory (IL-6, TNF-α and CRP), and mechanical stresses in eliciting chronic pain have been well-defined (McVinnie 2013). On the other hand, chronic pain conditions contribute to obesity through sedentary life style, depression and sleep disturbances (Bonakdar 2013). In fibromyalgia, several factors have been suggested to explain its relationship with obesity such as reduced physical activity, psychological disturbances, thyroid abnormalities, basal metabolic rate alteration and impaired somatotropic axis activity (Ursini et al. 2011).

Other dietary interventions have been associated with varying effects on pain. Non-substantial effects on pain were seen with the vegetarian diet assessed in the study of Azad et al. (2000), the Mediterranean and fasting therapies of Michalsen et al. (2005), the modified elimination diet of Lamb et al. (2011) and the integrative treatment approach of Michalsen et al. (2013). Two studies reported a significant drop of pain scores; one included the use of a vegetarian diet (Hostmark et al. 1993) and another adopted a vegan diet (Kaartinen et al. 2000); however, no data were reported to evaluate the effect sizes of change.

4.3.5 Psychological outcomes

The influence of both dietary interventions on depression and anxiety was shown to be negligible. To our knowledge, this is the first study that evaluates the effects of GFD on the psychological manifestations in fibromyalgia. On the other hand, the role of GFD in improving psychological disturbances of anxiety and depression among celiac disease patients has been investigated in several studies, given the increased prevalence of these manifestations among this population of patients (Hallert and Astrom 1982, Ciacci et al. 1998). The degree of benefit attained from the long-term adherence to GFD at the level of the psychological manifestations is unclear, with conflicting outcomes being reported (Addolorato et al. 2001, Fera et al. 2003, Pynnonen et al. 2005, Simsek et al. 2015).

An interesting study exploring the role of gluten in inducing psychological manifestations in patients with NCGS has been recently published (Peters et al. 2014). The authors concluded the presence of a depressive-inducing role for gluten among this group of patients which might explain their positive perception of this dietary intervention (Peters et al. 2014). However, results remain inconclusive given the exploratory nature of this study.

In fibromyalgia, the high prevalence of psychological disturbances is well-established (Thieme et al. 2004) and underlying pathophysiological mechanisms to explain this disturbances have been suggested such as the stress-induced HPA axis activation, altered sertoninergic and noradrenergic function and altered function of substance P, neurosteroids and cytokines (Gracely et al. 2012).

Behavioral weight loss therapy, in the study of Shapiro et al. (2005), was associated with a significant decrease in the BDI-II score from baseline (-8.2±7.1; ES: 0.7) and also a significant change in the BDI-II scores was achieved with the weight loss therapy in the study conducted by Senna et al. (2012) (-5.8, ES: 0.6), whereas in our study, HCD was associated with non-significant decrease in the BDI-II scores (-2.9±1.7, ES: 0.26). While patients recruited in the study conducted by Shapiro et al. (2005) and Senna et al.

(2012) can be classified as mildly depressed, our study sample consisted of severely depressed patients as reflected in the baseline BDI-II scores. Anxiety on the other hand was only evaluated by Shapiro et al. (2005) who reported a significant decrease in both STAI state and trait anxiety scores, reaching -7±10.2 and -7.5±8.8, respectively.

The negative impact of obesity on the psychological status of patients with fibromyalgia has been demonstrated in the correlation analysis conducted by Aparicio et al. (2014) and Arranz et al. (2012). However, obesity can be perceived as a factor among others that constitute a multidimensional model that is contributing to the precipitation of psychological disturbances in fibromyalgia, such as the biochemical changes in the brain, chronic pain, fatigue and sleep disturbances.

With respect to other dietary interventions among patients with fibromyalgia, the evaluation of their effect on depression was assessed in two studies, whereas anxiety was evaluated in only one study. Both, the vegan diet evaluated by Kaartinen et al. (2000) and in the integrative treatment approach of Michalsen et al. (2013), didn't lead to any significant change in depressive symptoms. Similar outcomes were seen with state and trait anxieties following the integrative treatment approach of Michalsen et al. (2013).

4.3.6 QOL

The effects of GFD and HCD on the mental and physical component summaries of the SF-12 were non-remarkable. In the two studies conducted by Rodrigo et al. (2013, 2014), the mental and physical component summaries of the SF-36 were significantly improved, indicating the beneficial outcomes of this dietary intervention among patients with fibromyalgia who present comorbid celiac disease or comorbid IBS and lymphocytic enteritis. However, the adoption of GFD by the general population of patients with fibromyalgia didn't seem to provide much benefit at the level of QOL.

In the study conducted by Shapiro et al. (2005), despite the significant improvements obtained at the level of physical functioning, changes in the QOL as evaluated by the Health Assessment Questionnaire-Standard Disability Index were non-significant. In a

meta-analysis evaluating the effect of weight loss on health-related quality of life in 53 randomized clinical studies, significant improvement in the physical health was concluded in contrast to the non-significant change in the mental health (Warkentin et al. 2014). Consistent with these conclusions, the outcomes of our study, although non-statistically significant, demonstrated that HCD was associated with a medium-sized improvement effect in the physical component summary in comparison to the negligible effect on the mental component summary.

Health-related QOL was evaluated in two studies evaluating other types of dietary interventions. In the first study, the use of vegan diet was linked to a significant improvement in the QOL as evaluated by the general health questionnaire (Kaartinen et al. 2000) and in the second study, significant improvements in the 8 subscales of the SF-36, excepting bodily pain, upon the adoption of a raw vegetarian diet were reported (Donaldson et al. 2001).

4.4 Limitations

Despite being the first randomized controlled trial that evaluates the effects of a gluten-free diet on the gluten sensitivity symptoms among patients with fibromyalgia, our pilot study was not void of some limitations. The lack of any validated scale that evaluates the severity of gluten sensitivity symptoms, both gastrointestinal and extraintestinal, has urged our investigation group to assemble a non-validated instrument represented in the clinical evaluation of a list of relevant symptoms and signs. Thus, it was not possible to assert the consistency or validity of the results generated by the instrument used to evaluate the primary outcome measure in our study.

Another limitation in the design of our study was the lack of neither investigator nor patient blinding. The nature of the dietary interventions adopted in our study made it unfeasible to implement blinding techniques. While it was impossible to blind the patient, the need of adequate orientation and follow up to the patients urged us to unblind the investigators as well.

Furthermore, the lack of sample size calculation, due to the absence of previous data related to our primary outcome measure, was another limitation preventing us from controlling the power of this study. The majority of previous studies evaluating the role of dietary interventions in fibromyalgia focused on the cardinal symptoms of fibromyalgia, i.e., pain, fatigue, physical functioning (FIQ) and psychological manifestations. However, none of these studies intended to evaluate the spectrum of gastrointestinal and extraintestinal manifestations mimicking gluten sensitivity symptoms.

The baseline characteristics of our study sample reveal the extreme severity of the various manifestations acquisitioned by this group of patients. In general, extremely symptomatic patients are usually referred to our research group from other departments, and thus, they might not be representative of the general population of patients with fibromyalgia where cases with less severe manifestations can be found.

4.5 Conclusions

1) Both dietary interventions, GFD and HCD, were associated with similar beneficial outcomes, which were time-significant, in reducing gluten sensitivity symptoms. However, the differences between the two interventions were non-significant.

2) The high frequency of food intolerance in fibromyalgia suggested a possible role for hypersensitivity to certain dietary components such as gluten in the occurrence of the gastrointestinal manifestations. However, the non-significant differences between the two interventions investigated in our study do not support the presence of a specific role of gluten in eliciting these manifestations in our sample of patients.

3) Gastrointestinal manifestations are highly prevalent among patients with fibromyalgia. However, they are generally overlooked in studies that are not specifically dedicated to evaluate these manifestations. Considering the high prevalence and disabling nature of the gastrointestinal manifestations which contribute to an impaired quality of life among patients with fibromyalgia, studies directed to evaluate such manifestations are needed. Our study is the first randomized clinical trial that aimed at evaluating the improvement of these manifestations, among patients with fibromyalgia, in response to two types of dietary interventions.

4) Equivalent effects for both dietary interventions were obtained in improving secondary outcome measures. With respect to anthropometric outcomes, changes in the BMI were shown to be dependent of the basal BMI; clinically, the effect sizes in both groups were small except for waist circumference that was linked to medium effect size of change with HCD only. Although inter-group differences in the FIQR scores were non-significant, intragroup differences were clinically relevant with medium effect sizes of change being achieved in both groups. Also, clinically relevant improvement in the physical component summary of the SF12 was achieved only among patients placed on HCD.

5) Both dietary interventions were safe and well-tolerated.

6) The results obtained in our study lead to the arousal of other interesting research questions. As such, it would be of interest to investigate the role of a restriction diet with a broader range of restricted dietary content, such as a FODMAP-free diet. Another interesting research question would be the estimated prevalence of non-celiac gluten or wheat sensitivity among patients with fibromyalgia.

7) The role of dietary therapies in the treatment of fibromyalgia is important and requires further investigation. To date the lack of well-designed controlled clinical trials investigating the role of dietary interventions in fibromyalgia avoids the adoption of a clear recommendation, backed by an adequate level of evidence, to adopt such therapies for the treatment of fibromyalgia.

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Annex 1: HOJA DE INFORMACIÓN AL PACIENTE Y CONSENTIMIENTO INFORMADO

TÍTULO DEL ESTUDIO:

"ENSAYO CLÍNICO ABIERTO Y ALEATORIZADO COMPARANDO DIETA SIN GLUTEN CON DIETA HIPOCALÓRICA EN PACIENTES CON FIBROMIALGIA"

Se le pide que participe en esta investigación. Antes de decidir si desea participar, es importante que entienda por qué se realiza, cómo se utilizarán sus datos, lo que implicará el presente estudio y los posibles efectos beneficiosos, riesgos y molestias que puedan derivarse. Tómese el tiempo que necesite para leer detenidamente la siguiente información y comentarla con su médico, si lo desea.

¿CUÁLES SON LOS ANTECEDENTES Y EL PROPÓSITO DEL ESTUDIO?

Se sabe todavía muy poco sobre la evolución de la fibromialgia a lo largo del tiempo, así como sobre la respuesta a los tratamientos a largo plazo y los factores que pueden modificar la evolución de la enfermedad. Se han ensayado diferentes abordajes terapéuticos tanto farmacológicos como no farmacológicos (psicoterapia, fisioterapia, musicoterapia, técnicas de relajación...) con diferentes resultados. Dentro de los factores susceptibles de modificar el curso de la enfermedad uno de los más accesibles puede ser la nutrición. Los escasos estudios realizados al respecto apuntan a las dietas que llevan al normopeso del paciente como las más beneficiosas.

El estudio actual pretende comparar dos tipos de dietas y estudiar su efecto en la sintomatología de aquellos pacientes con fibromialgia que además tengan una sintomatología digestiva similar a la de enfermos celíacos adultos. Asimismo, nos interesa saber cual es su calidad de vida a lo largo de este período. Todo ello nos ayudará a comprender mejor su enfermedad y los problemas que plantea.

¿TENGO QUE PARTICIPAR?

Es usted quien decide si desea participar o no. Aunque no desee participar en este estudio, no saldrá perjudicado y recibirá el tratamiento y la asistencia médica a los que tiene derecho. Si decide participar, se le pedirá que firme este consentimiento informado, aunque podrá retirarse del estudio en cualquier momento. Esto no afectará a la asistencia que reciba.

¿QUÉ ME OCURRIRÁ SI PARTICIPO?

El estudio exige 7 visitas y una revisión por teléfono. Las primeras serán quincenales aproximadamente y posteriormente se espaciarán en 4 ó 6 semanas hasta la finalización del estudio. En total la duración del estudio será de 6 meses.

En la primera visita se comprobará si usted reúne las características requeridas para participar en el estudio y, en caso afirmativo, se le dará información verbal y escrita que le ayude a decidir si desea

o no quedar incluido en el mismo. De ser así, se le realizarán varias evaluaciones con 3 análisis de sangre rutinarios y varias pruebas psicológicas, se le preguntará acerca del estado de su salud, las enfermedades y los tratamientos que Vd. tenga prescritos.

¿QUÉ TENGO QUE HACER?

Debe estar dispuesto a realizarse las oportunas extracciones sanguíneas, cumplimentar los cuestionarios que se le entreguen y a responder a las preguntas que los investigadores le realicen en relación a su enfermedad, así como a asistir a las visitas programadas.

¿CUÁLES SON LOS POSIBLES EFECTOS SECUNDARIOS, RIESGOS Y MOLESTIAS POR PARTICIPAR?

Las únicas molestias derivadas de su participación en este estudio serán las propias de la técnica de extracción sanguínea, las de acudir a las visitas indicadas y rellenar algunos cuestionarios.

¿CUÁLES SON LOS POSIBLES EFECTOS BENEFICIOSOS DE PARTICIPAR?

Ante todo para el propio paciente se espera que cualquiera de las dietas mejore su sintomatología de la fibromialgia ya sea por conducir al peso normal de la persona o por la eliminación de la causa de una celiaquía subclínica.

TRATAMIENTO DE SUS DATOS PERSONALES

El derecho de acceso, rectificación y cancelación a sus datos personales, sus resultados en las pruebas objetivas y aquellos referentes a su salud cumplirán en todo momento, por parte de los responsables de su custodia y tratamiento fines de investigación, con lo establecido por la Ley Orgánica 15/1999, de 13 de diciembre, de protección de datos de carácter personal y el Real Decreto 1720/2007, de 21 de diciembre.

¿CON QUIÉN PUEDO CONTACTAR SI NECESITO MÁS INFORMACIÓN O AYUDA?

Si necesita más información que no le ha podido proporcionar el médico que le atiende en este estudio puede contactar con:

Dra. Elena Pita Calandre

Instituto de Neurociencias
Universidad de Granada
Avda. Madrid, 11
18012 – Granada

DECLARACIÓN DE CONSENTIMIENTO INFORMADO

He recibido información verbal del estudio y he leído la información escrita de este documento.

He tenido la oportunidad de comentar el estudio y realizar preguntas.

Consiento en participar en el estudio y soy consciente de que mi participación es completamente voluntaria.

Entiendo que me puedo retirar del estudio en cualquier momento sin que afecte a mi atención médica futura.

Recibiré una copia firmada y fechada de este documento de información y consentimiento.

Participante: _____ con DNI _____

(Nombre del paciente)

lo firmo el día ____ de _____ de 201__

Firma del/a paciente:

Investigador que explicó el consentimiento

_____ con DNI _____

(Nombre del investigador/a)

Firma del investigador

Annex 2: Chronogram of the study

Week number	**-2**	**0**	**2**	**4**	**8**	**12**	**18**	**24**
Visit number	1	2	3 (Telephone)	4	5	6	7	8
Gluten-sensitivity symptoms evaluation	X			X	X	X	X	X
Medical history, demographic data and informed consent	X							
Complete blood count, biochemistry and serology	X							X
Diet		Assigning diets	Doubts clarification					
Anthropometric measurements	X			X	X	X	X	X
FIQ-R	X			X	X	X	X	X
PSQI	X			X	X	X	X	X
BPI	X			X	X	X	X	X
BDI-II	X			X	X	X	X	X
STAI	X			X	X	X	X	X
SF-12	X			X	X	X	X	X
PGS-S	X			X	X	X	X	X
PGI-I				X	X	X	X	X
Adverse events			X	X	X	X	X	X