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DOUTORAMENTO EM NUTRIÇÃO CLÍNICA

Effects of a nutritional intervention poor in potentially inflammatory components in patients with Fibromyalgia

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Declaro que colaborei ativamente na definição dos objetivos e métodos de todos os manuscritos que constituem esta tese, conduzi a implementação do estudo, realizei a análise dos dados e colaborei na sua interpretação. Fui responsável pela redação da versão inicial e participei ativamente na elaboração das versões finais de todos os manuscritos aqui apresentados.

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Acronyms and abbreviations

AGEs – Advanced glycated end product

BCAA - Branched-chain amino acids

BDI – Beck's depression inventory

BDNF – Brain-derived neurotrophic factor

BMI – Body mass index

BPI – Brief pain inventory

BSQ –Body shape questionnaire

CH – Carbohydrates

CNS – Central nervous system

hs-CRP – high sensitive – C-Reactive protein

EQ-5D – Five dimension Euro quality of life

EULAR - European League Against Rheumatology

ESR – Erythrocyte sedimentation rate

FFQ – Food frequency questionnaire

FIQR – Fibromyalgia impact questionnaire revised

FM – Fibromyalgia

FODMAPs – Fermentable oligo-, di- and monosaccharides, alcohol and polyols

FOSQ – Functional outcome of sleep questionnaire

FSS – Fatigue severity survey

FSQ – Functional status questionnaire

GABA – Gamma-aminobutyric acid

GI – gastrointestinal

GLM – General linear model

HAQ – Health assessment questionnaire

HDAC - Histone deacetylase

^{1}H NMR - Hydrogen nuclear magnetic resonance spectroscopy

HPA – Hypothalamus Pituitary Adrenal axis

IBS – Irritable bowel syndrome

IBS-SSS – Irritable bowel syndrome – severity scoring system

IL – Interleukin

INF-γ – Interferon-gamma

LHBT – lactulose hydrogen breath test

MCP1 - Monocyte chemoattractant protein 1

MPI – multidimensional poverty index

MUFA - monounsaturated fatty acid

NCGS – nonceliac gluten sensitivity

NGF - nerve growth factor

NF-κB - Nuclear factor kappa B

NREM – Non-rapid eye movement

NSAIDs – Non-steroidal anti-inflammatory drugs

PDI-I – Patient dignity inventory

PRO – Patient Reported Outcomes

PSQI – Pittsburg Sleep Quality Inventory

PUFA - polyunsaturated fatty acid

QOL – Quality of life

RCT – Randomized controlled trial

REM – Rapid eye movement

RNS - Reactive nitrogen species

ROS - Reactive oxygen species

RSQ-D – Daily restorative sleep questionnaire

SAMe - S-adenosylmethionine

SCFA – Short chain fatty acids

SD – Standard deviation

SF-36 – Short-form Health Survey 36

SF-36_Mental – Short-form Health Survey 36 for Mental Aspect

SF-36_Physical - Short-form Health Survey 36 for Physical Aspect

SIBO – Small intestinal bacterial overgrowth

SP - Substance P

SRSBQ – Sleep related and safety behavior questionnaire

STAI – State trait anxiety inventory

Th – T helper

TMAO – Trimethylamine N-oxide

TNFα – Tumor necrosis factor alpha

TP – Tender Points

Treg – T regulater

TSS – Tiredness symptoms scale

UCT – Unrandomized controlled trial

VAS – Visual Analogic Pain Scale

VAS_GI – Visual Analogic Scale for Gastrointestinal Symptoms

VCAM-1 - Vascular cell adhesion molecule 1

WPI – Widespread pain index

WHO – World Health Organization

Abstract

Fibromyalgia (FM) is a chronic disease, whose main symptoms are pain, tiredness, poor sleep quality, and gastrointestinal disorders. Its prevalence in the world and in Portugal is high, and the fact that there is no effective medical therapy increases the clinical interest in its investigation.

Although its etiology is unknown, some authors refer to the existence of a dysfunction in the central nervous system afferent pain pathways in FM patients, which translates into an amplification of the pain sensation. On the other hand, the presence of low-grade systemic inflammation, which was also identified in these patients through a high serum concentration of interleukin (IL) 8 and IL-6, also promotes the sensation of generalized pain. Additionally, the relationship of FM with the possible existence of dysbiosis has also been studied, reporting the presence of Small Intestinal Bacterial Overgrowth (SIBO). The symptoms of SIBO, namely constipation or diarrhea, abdominal pain, bloating and flatulence, are very common in FM patients.

Since pharmacological therapy cannot completely resolve this disease, nutritional strategies emerge as a treatment hypothesis. However, according to the literature, the effect of nutritional interventions on FM remains controversial. In a Systematic Review carried out by our team, we found that the quality of published studies was low and with a high risk of bias. Additionally, there seemed to be a need for a nutritional intervention that encompasses a dietary pattern, rather than the exclusion of isolated nutrients or foods, and that would integrate all the possible etiopathogenic mechanisms that may explain the disease, namely low-grade inflammation and intestinal dysbiosis. Thus, this thesis aimed to analyze the effects of a diet with anti-inflammatory properties and low in foods rich in FODMAPs, on the main manifestations of FM, namely pain, fatigue, gastrointestinal changes, sleep quality and quality of life.

This Randomized Controlled Trial (NCT04007705) included 46 FM female patients, recruited in *Instituto Português de Reumatologia*. Intervention group (n=22) adopted an anti-inflammatory diet for 3 months, excluding gluten, dairy, added sugar and ultra-processed

foods, along with a low FODMAPs diet in the first month. Control group (n=24) followed World Health Organization (WHO) general healthy eating recommendations. Before and after intervention, participants were assessed regarding pain, fatigue, gastrointestinal symptoms, quality of sleep and quality of life, through: Revised Fibromyalgia Impact Questionnaire (FIQR), Visual Analogue Pain Scale (VAS), Visual Analogue Scale from gastrointestinal symptoms (VAS GI), Brief Pain Inventory (BPI), Pittsburg Sleep Quality Index (PSQI), Fatigue Severity Survey (FSS) and The Short Form Health Survey (SF-36). A blood sample was collected and High-sensitive C-Reactive Protein (hs-CRP) and Erythrocyte Sedimentation Rate (ESR) were quantified.

Anthropometric data, namely height, weight, and waist circumference, were collected at the beginning and end of the intervention. The socio-demographic, clinical, and lifestyle characteristics of the participants were also collected. The food intake of the participants was evaluated at the beginning, through food recall of the previous 24 hours, and 3-day food diaries were applied, fortnightly, during the intervention period.

Paired Samples T-Test/Wilcoxon and independent samples T-Test/Mann-Whitney were used to compare variables between groups.

After the intervention, there was an improvement in the scores of all the questionnaires applied in the intervention group. Although of lesser magnitude, in the control group there was an improvement in some of the parameters evaluated, namely gastrointestinal symptoms, fatigue and sleep. Compared to control group, the intervention group showed improvements in respect to pain and functional repercussion (FIQR -19.9 ± 18.8 vs -2.2 ± 16.1; p=0.001; VAS -2.3 ± 2.5 vs -0.04 ± 2.1; p=0.002; BPI -3.8 ± 4.1 vs -1.1 ± 2.6; p=0.011), gastrointestinal alterations (VAS_GI -2.0 ± 0.9 vs -0.9 ± 1.3; p=0.002); fatigue (FSS -1.1 ± 1.2 vs -0.5 ± 1.0; p=0.042), sleep quality (PSQI -3.5 ± 4.6 vs -1.2 ± 2.6; p=0.048), and quality of life (SF36 10.2 ± 11.2 vs 3.6 ± 10.4; p=0.045) in the end of the intervention. Inflammatory biomarkers (hs-CRP, ESR) did not change in both groups.

The intervention was beneficial in the intervention group, regardless of age, disease duration, body mass index variation and body fat changes between baseline and post-intervention. The anti-inflammatory diet could potentially have reduced low-grade inflammation, characteristic of FM, promoting the reduction of pain associated with the disease. Moreover, low FODMAPs

diet may have possibly reduced SIBO and optimized intestinal microbiota, allowing a greater efficacy of the posterior anti-inflammatory approach. Additionally, the improvement in intestinal function, and possibly in microbiota composition, may have potentiate the better absorption of vitamins, minerals, and other food components, which may have contributed to the fatigue reduction.

The present study allows us to conclude that an anti-inflammatory and low FODMAPs diet improved patient reported outcomes in FM patients, which may represent a relevant complement to the pharmacological therapy.

Resumo

A fibromialgia (FM) é uma doença crónica, cujos principais sintomas são dor, cansaço, alterações do sono, e alterações gastrointestinais. A sua prevalência no mundo e em Portugal é elevada, e o facto de não existir terapêutica médica eficaz, faz aumentar o interesse clínico na sua investigação.

Apesar de não se conhecer a sua etiologia, alguns autores referem a existência de uma disfunção nas vias aferentes da dor do sistema nervoso central nos doentes de FM, o que se traduz por uma amplificação da sensação da dor. Por outro lado, a presença de inflamação sistémica de baixo grau, que foi também identificada nestes doentes através de uma elevada concentração sérica de interleucina (IL) 8 e IL-6, promove também a sensação de dor generalizada. Adicionalmente, a relação da FM com a possível existência de disbiose também tem vindo a ser estudada, relatando-se o sobrecrescimento das bactérias do intestino delgado (SIBO – *Small Intestinal Bacterial Overgrowth*). Os sintomas do SIBO, nomeadamente, obstipação ou diarreia, dor abdominal, distensão abdominal e flatulência, são muito comuns nos doentes de FM.

Dado que a terapêutica farmacológica parece não resolver por completo as manifestações desta doença, as estratégias nutricionais surgem como uma oportunidade de tratamento. Contudo, de acordo com a literatura, o efeito das intervenções nutricionais na FM permanece controverso. Numa Revisão Sistemática que realizámos, verificámos que a qualidade dos estudos publicados era baixa e com elevado risco de viés. Adicionalmente, parecia faltar uma intervenção nutricional holística, ao invés da exclusão de nutrientes ou alimentos isoladamente, e que pudesse integrar os possíveis mecanismos etiopatogénicos explicativos da doença, nomeadamente a inflamação de baixo grau e a disbiose intestinal. Assim, esta tese teve como objetivo analisar os efeitos de uma dieta com propriedades anti-inflamatórias e pobre em alimentos ricos em FODMAPs, nas principais manifestações da FM, nomeadamente dor, fadiga, alterações gastrointestinais, qualidade do sono e qualidade de vida.

Este ensaio clínico controlado e randomizado (NCT04007705) incluiu 46 pacientes do sexo feminino com FM, recrutados no Instituto Português de Reumatologia. O grupo intervenção

(n=22) adotou uma dieta anti-inflamatória por 3 meses, excluindo glúten, laticínios, açúcar de adição e alimentos ultra-processados. No primeiro mês de intervenção acresceu à dieta anti-inflamatória, a restrição em FODMAPs. O grupo controlo (n=24) seguiu as recomendações gerais para uma alimentação saudável, de acordo com a Organização Mundial da Saúde. Antes e após a intervenção, os participantes foram avaliados relativamente a dor, fadiga, sintomas gastrointestinais, qualidade de sono e qualidade de vida, através dos seguintes questionários: *Revised Fibromyalgia Impact Questionnaire* (FIQR), Visu*al Analogue Pain Scale* (VAS), *Visual Analogue Scale from gastrointestinal symptoms* (VAS GI), *Brief Pain Inventory* (BPI), *Pittsburg Sleep Quality Index* (PSQI), *Fatigue Severity Survey* (FSS) and *The Short Form Health Survey* (SF-36). Foram quantificadas a Proteína C-Reativa de Alta Sensibilidade (hs-CRP) e a Velocidade de Sedimentação (ESR) através da colheita de uma amostra de sangue.

Dados antropométricos e de composição corporal foram recolhidos no início e no final da intervenção. Foram também recolhidas as características socio-demográficas, clínicas, e de estilo de vida, dos participantes. Foi avaliada a ingestão alimentar dos participantes no momento inicial, através da recordação alimentar das 24 horas anteriores, tendo sido ainda aplicados diários alimentares de 3 dias, quinzenalmente, durante o período da intervenção.

Utilizaram-se os testes T-Test/Wilcoxon para amostras emparelhadas e T-Test/Mann-Whitney para amostras independentes para comparar as variáveis entre os grupos.

Após a intervenção, verificou-se uma melhoria nos scores de todos os questionários aplicados no grupo intervenção. Embora de menor magnitude, no grupo controlo observou-se uma melhoria em alguns dos parâmetros avaliados, nomeadamente sintomas gastrointestinais, fadiga e sono. Comparativamente com o grupo controlo, o grupo intervenção apresentou melhorias relativamente à dor e repercussão funcional (FIQR -19.9 ± 18.8 vs. -2.2 ± 16.1; p=0.001; VAS -2.3 ± 2.5 vs. -0.04 ± 2.1; p=0.002; BPI -3.8 ± 4.1 vs. -1.1 ± 2.6; p=0.011), alterações gastrointestinais (VAS_GI -2.0 ± 0.9 vs. -0.9 ± 1.3; p=0.002); fadiga (FSS -1.1 ± 1.2 vs. -0.5 ± 1.0; p=0.042), qualidade do sono (PSQI -3.5 ± 4.6 vs. -1.2 ± 2.6; p=0.048), e qualidade de vida (SF36 10.2 ± 11.2 vs. 3.6 ± 10.4; p=0.045) no final da intervenção.

Relativamente aos biomarcadores inflamatórios (hs-CRP, ESR), não foram observadas diferenças em ambos os grupos.

A intervenção foi benéfica no grupo intervenção, independentemente da idade, duração da doença, variação do índice de massa corporal e alterações da gordura corporal entre o início e o final da intervenção. A dieta anti-inflamatória pode potencialmente ter reduzido a inflamação de baixo grau, característica da FM, promovendo a redução da dor associada à doença. Além disso, a dieta com baixo teor de FODMAPs pode ter possivelmente reduzido o SIBO e otimizado a microbiota intestinal, permitindo maior eficácia da abordagem anti-inflamatória posterior. Adicionalmente, a melhoria na função intestinal, e possível melhoria da composição da microbiota intestinal, pode ter potencializado a melhor absorção de vitaminas, minerais e outros componentes nutricionais, o que pode ter contribuído para a redução da fadiga.

O presente estudo permite concluir que uma dieta anti-inflamatória e baixa em FODMAPs melhorou as manifestações da doença em pacientes com FM, o que pode representar um complemento relevante à terapêutica farmacológica.

1. Theoretical framework

1. Theoretical framework

This chapter presents the theoretical framework of this thesis. It is divided into three parts. In the first part, the main characteristics of FM are presented, including symptoms, diagnosis, prevalence, and medical therapy usually applied. The second part strive for the identification of the main metabolic and physiologic mechanisms associated with the etiopathology of FM, namely from the point of view of the central nervous system, intestinal microbiota, and inflammation. Finally, the third part reveals all dietary interventions carried out in FM patients up to the date.

1.1. Fibromyalgia characterization and prevalence

Fibromyalgia (FM) is a chronic non degenerative disease, which etiology remains unknown [1, 2]. It is characterized by generalized chronic musculoskeletal pain, accompanied by other symptoms such as chronic fatigue, asthenia, anxiety, depression, and changes in sleep pattern [1-3]. Moreover, patients also commonly have associated pain co-morbidities or other conditions, such as lower back pain, muscle stiffness, restless leg syndrome and leg cramps, headache, migraine, palpitation [4] and temporomandibular disorders [2, 3].

The disease diagnosis is substantially clinic. According to the Rome III criteria determined by the American College of Rheumatology, the diagnosis includes palpation pain present in at least 11 of the 18 Tender Points, axial and bilateral pain, above and below the waist, and described for a period exceeding 3 months [5]. So far, there are no specific laboratory tests to confirm FM diagnosis.

The prevalence of FM worldwide has been reported between 2 and 4%, affecting mainly women at any age [6, 7]. In Europe, an epidemiological study conducted in 2009 in five countries (Portugal, Spain, France, Italy and Germany), with a total sample of 4517 individuals, applied the London FM Epidemiology Study Screening Questionnaire by telephone interview. The overall prevalence was 2.9% (95% CI 2.4-3.4), being 2.1% (95% CI 2.0-2.2) in men and 3.6% (CI 95% 3.5-3.7) in women [8]. According to this study, in the Portuguese population the

prevalence was 3.6% (IC 95% 2.0-5.2), being 2.3% (IC 95% 2.1-2.5) in men and 5.1% (IC 95% 4.8-5.4) in women [8]. According to the *Instituto Nacional de Estatística,* Portugal had in 2021, a population of 10.344.802 people, which would translate to more than 370.000 people with FM in the country.

The medical therapeutic protocol involves intervention with muscle relaxants, analgesics, antidepressants and anxiolytics. However, FM patients continue to experience moderate pain and a change in its regulation mechanisms [9].

FM is a very debilitating disease that interferes with work ability, daily activities and personal and family relationships. Quality of life and health status in FM patients is worst compared to other chronic diseases, including osteoarthritis, rheumatoid arthritis, systemic lupus erythematosus, myocardial infarction, chronic obstructive pulmonary disease, congestive heart failure, hypertension, and diabetes [10].

Given the lack of knowledge about its etiopathogenesis, many complementary non-pharmacological approaches have emerged. However, studies in general are of poor quality, with small samples and often without control groups [11]. The European League Against Rheumatology (EULAR) Society classified as strong, weak, or not recommended some of the approaches used for these patients, as a result of a systematic review. The exercise regular practice was considered a strong recommendation, although the distinction between aerobic and strengthening was not evident in the light of published studies. Given the poor quality of the studies found, the practice of mindfulness-based stress reduction, meditative movement therapies, physical therapies, acupuncture, and hydrotherapy were considered to be weak recommendations. Due to the lack of effectiveness and/or low study quality, EULAR does not recommend biofeedback therapy, hypnotherapy, massage, capsaicin and S-adenosylmethionine (SAMe) supplementation. EULAR also takes a position of “strong against” evaluation for chiropractic therapy [12].

FM imposes significant economic burden, as patients often have a high prevalence of work loss. In fact, approximately 56-60% of patients quit their jobs or claim for limitations in labour, and the annual days missed from work are 23.2 to 32.5 days per year [13]. However, there is little data on the economic real impact of FM. The existing studies refer to different countries, some including in their samples, patients with conditions other than FM, and different

hospitalization times. As it does not represent a homogeneous sample, it is difficult to draw general conclusions.

1.2. Fibromyalgia etiopathogenesis

Despite the unknown disease etiology, FM symptoms appear to be associated with several metabolic imbalances, notably with respect to changes in the hypothalamus-pituitary-adrenal (HPA) axis and consequent increase in cortisol [14, 15] and central nervous system (CNS) activation, with glia cells stimulation in cerebrospinal fluid [16]. In this sense, stress would be a likely trigger for the symptomatology of the disease [17].

Additionally, the link between the gut microbiota and CNS is well described in the literature [18, 19], so gut optimization would also be important. In fact, some authors argue that the persistence of the FM symptoms is associated with possible changes in the intestinal microbiota [2], with consequent existence of Small Intestinal Bacterial Overgrowth (SIBO) [20-22], intestinal hyperpermeability and dysbiosis.

On the other hand, some authors suggest a presence of low-grade inflammation in FM patients, identified by an elevation in interleukin (IL)-8 and IL-6 [1]. The association between low-grade inflammation and dysbiosis is already known [23], and intestinal inflammation has been described by some authors [2, 24-26].

1.2.1. Fibromyalgia and central nervous system

The perception of pain results from the activation of certain sensory receptors – nociceptors - specialized in detecting a stimulus of damage to the body. In the case of FM, a descending and ascending inhibitory pathway dysfunction has been suggested [27], combined with a possible change in neurochemical balance in CNS [28].

FM patients appear to have an increase in cerebrospinal fluid levels of excitatory neurotransmitters such as glutamate, substance P (SP), nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), and a decrease in inhibitory neurotransmitters, such as serotonin, dopamine and noradrenaline, which will potentially facilitate signal transmission,

leading to amplified pain perception [28]. In its turn, SP, glutamate and BDNF seam to activate glia cells through receptors localized on microglia and astrocytes. This activation release pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), IL-1β and IL-8, and also BDNF, NGF, glutamate and SP. Activation of glia cells can further increase pain amplification and this could be implicated in the altered pain modulation in FM patients [29].

Glia cells could also be activated through blood-borne, pro-inflammatory cytokines released by peripheral immune cells and transported across blood-brain barrier by a special transport mechanism. In fact, increased levels of serum IL-8 were reported in FM patients [1].

1.2.2. Fibromyalgia and intestinal microbiota

Several authors advocate the presence of an intestinal inflammation in FM patients, derived from an alteration of the intestinal microbiota, with consequent intestinal dysbiosis and hyperpermeability. Changes in barrier function are related to an increase in pro-inflammatory cytokines, namely TNF-α, IL-1β and IL-13, expressed in chronic intestinal inflammation [2]. Chronic inflammation appears to result from an inadequate immune response as a consequence of genetic predisposition, as well as changes in the intestinal microbiota. On the other hand, an insufficient response to a stimulus of a bacterium results in an insufficient immune response to pathogens [30].

Dysbiosis seems to influence the occurrence of systemic and chronic metabolic diseases, possibly by activating the expression of inflammatory cytokines and immune cells, namely Thelper (Th) lymphocytes 1, Th2 and Th17, and Tregulators (Treg) [31]. Metabolic syndrome and obesity [32, 33], neuropsychiatric diseases and CNS disruption [34, 35], autoimmune diseases [36], intestinal inflammatory diseases [32, 37] and rheumatic diseases [38] are all associated to dysbiosis. In fact, in 1991 George Triadafilapoulos and Robert Simms published an article reporting a high prevalence of gastrointestinal symptoms suggestive of Irritable Bowel Syndrome (IBS) in FM patients [39]. More recently, other authors have also identified similarities between FM symptoms and IBS symptoms [2, 3, 40], namely nausea, vomiting, dyspepsia, sleep changes and chronic fatigue.

At the same time, in the presence of dysbiosis, serotonin and gamma-aminobutyric acid (GABA) production will be compromised, which in the long term is associated with depression [41], another FM common symptom.

In a study conducted by Malatji and colleagues, several urine metabolites were identified by Hydrogen nuclear magnetic resonance spectroscopy (^{1}H NMR) in FM patients suggesting changes in the intestinal microbiota, namely: 1) hyperuric acid; 2) 2-hydroxyisobutyrate acid, associated with the presence of Faecalibacterium Prausnitzii, a commensal bacterium; 3) lactic acid; 4) taurine, succinate acid and Trimethylamine N-Oxide (TMAO) [42].

Associated to dysbiosis, the presence of Small Intestinal Bacterial Overgrowth (SIBO) was identified as frequent in these patients [20-22], as SIBO appears to increase the exposure of immune system cells to antigens in the intestinal lumen, thereby causing immune modulation [42].

The determination of SIBO can be performed by applying the lactulose hydrogen breath test (LHBT) after oral ingestion of 10 grams of lactulose. The diagnosis is considered when two hydrogen peaks are present, each with at least 10 particles per million [43]. In a double-blind study developed by Pimentel and colleagues [22] in a population of 42 FM patients and 111 IBS patients, LHBT was applied to all patients. The presence of SIBO was diagnosed in all FM patients and in 84% of IBS patients, comparatively with only 20 % in the control group.

The treatment of SIBO involves minimizing the intake of foods rich in fermentable oligo-, di- and monosaccharides, alcohols and polyols (FODMAPs) for a period of between 4 and 6 weeks, as these are mainly absorbed in the colon, forming hydrogen (H2) and methane (CH4), which generates flatulence, bloating and abdominal pain, as well as diarrhea [20]**.** Necessarily, this intervention involves avoiding all dairy products; all cereals except rice; the cashew nuts; all fruits except bananas, citrus fruits, pineapples, berries, strawberries and kiwi; all vegetables except pumpkin, kale, lettuce, tomatoes, carrots and cucumbers.

A meta-analysis conducted by Marsh and colleagues [20] supports the effectiveness of a diet with a low intake of FODMAP-rich foods in the treatment of gastrointestinal symptoms present in IBS. The symptoms considered were abdominal pain and distension, constipation, diarrhea and flatulence.

1.2.3. Fibromyalgia and inflammation

Although it is not considered an inflammatory disease, it has been reported the presence of a low-grade inflammation in FM, characterized mainly by the increase of IL-8, as shown by Mendieta and colleagues in a Systematic Review [1].

Inflammation is a non-specific central component of the innate immune system of humans, that can be triggered by pathogens, damaged cells and toxic compounds [44]. It is essential to the body's adaptive processes and defence. Acute inflammatory response is characterized by cellular and molecular events and interactions that prevent injury or infection, which contributes to restoration of tissue homeostasis and resolution of the acute inflammation. However, uncontrolled acute inflammation may become chronic, which over a long term and if persistent, may give place to a pathology. There are several events arising from chronic low grade inflammation process, such as vascular permeability changes, leukocyte recruitment and accumulation, and inflammatory mediator release [44].

Chronic low grade inflammation is associated to the development of a variety of chronic inflammatory diseases [44, 45] such as cancer [46], autoimmune diseases [47], obesity and metabolic syndrome [45], rheumatic diseases [48], among others. Some studies point to an association between FM and intestinal inflammation [2, 24-26], with an increase of serum pro-inflammatory cytokines, such as IL-1β, IL-6, IL-8 and IL-10 and TNF-α [49] in these patients. Romano and colleagues identified a significant change in various biochemical parameters in FM patients, including high C-Reactive Protein (CRP), TNF-α, IL-1β, IL-6 and IL-8, and low levels of serotonin, free fatty acids and polyunsaturated fatty acids (PUFAs) [15]. A study conducted by Kadetoff and colleagues determined the presence of elevated levels of IL-8, but not IL-1β, in cerebrospinal fluid in FM patients [16]. These results were supported by other authors, such as Wang and colleagues [50], Mandieta and colleagues [1] and Bazzichi and colleagues [49].

In turn, dysbiosis appears to increase the exposure of immune cells to antigens in the intestinal lumen, thereby causing immune modulation and expression of pro-inflammatory cytokines [37]. In fact, a meta-analysis performed in 2011 points to increased production of pro-inflammatory cytokines, particularly IL-8 and IL-6, in FM patients [51]. This suggests the

presence of low-grade chronic inflammation that perpetuates the symptomatology of the disease.

1.3. Dietary interventions in fibromyalgia

As pharmacological therapy seems not to completely resolve the symptoms of the disease [2, 9], dietary approaches emerge as an important opportunity. However, according to the literature, the effect of nutritional interventions on FM remains controversial. A Systematic Review conducted by our team in 2018 found that the few clinical trials to date were of poor quality and high risk of bias [52]. However, the results were promising for a hypocaloric diet [53, 54], a raw vegetarian diet [55] or a low FODMAPs diet [56], in respect to improvement of pain and functional repercussion in FM patients [52]. The summary of results evaluating various dietary interventions in fibromyalgia is shown in table 1.

Table 1. Summary dietary interventions in fibromyalgia patients

Reference	Study Design and Participants	Intervention	Outcome Measures	Results
[56]	UCT Female FM patients (n=38) Age: 51 ± 10 Y	Diet low in FODMAPs (LFD) 4 weeks (31 women completed the intervention)	- Questionnaires: FSQ, FIQR, IBS-SSS, EQ-5D, VAS (abdominal pain and somatic pain); - evaluation of satisfaction with diet;	<u>Comparison before and after the intervention:</u> - ↓ pain associated with FM, fatigue, gastric pain and intestinal changes after 4 weeks ($p < 0.01$) - ↓GI symptoms ($p<0.01$) - ↑ mobility and ↓ discomfort in T2 ($p < 0.05$) - no significant differences in quality of life
[57]	RCT Diet Group: n=35 female FM patients Median age: 52 (36-66) Y Control Group: n=40 female FM patients Median age: 53 (32-65) Y	Gluten-free diet (GFD) VS Hypocaloric Diet (HD); 6 months	- Anthropometric data: weight, BMI, waist perimeter - Biochemical analyzes - List of NCGS symptoms (GI symptoms, extraintestinal and FM-like) - Questionnaires: FIQR, BPI, PSQI, BDI, STAI, SF-12, PGI-I	<u>Comparison before and after the intervention:</u> - no significant differences in pain associated with FM and GI, extraintestinal and FM-like symptoms in GFD and HD <u>Comparison between groups:</u> - no significant differences
[58]	Randomized Crossover Trial FM patients (n=20; 19 female) Age: 48.9 ± 12.3 Y	Khorosan wheat-based replacement diet (KD) VS Wheat normal diet (WD)	- Anthropometric: BMI - Questionnaires: WPI, WPI-SS, FOSQ, FIQ, FSS, TSS, SRSBQ, RSQ-D	<u>Comparison before and after the intervention (KD):</u> - ↓ pain associated with FM ($p < 0.05$), widespread pain ($p < 0.05$) - improve in functional outcome of sleep ($p < 0.05$) <u>Comparison between groups:</u> - ↓ pain associated with FM ($p < 0.05$) in KD compared to WD

RCT – Randomized controlled trial; UCT – Unrandomized controlled trial; FIQR – Revised Fibromyalgia impact questionnaire; FSQ – Functional status questionnaire; IBS-SSS – Irritable bowel syndrome – severity scoring system; EQ-5D – Five dimension Euro quality of life; VAS – Visual analogue pain scale; BPI - Brief ain inventory; PSQI - Pittsburg sleep quality index; BDI – Beck's depression inventory; STAI – State trait anxiety inventory; SF36/12 – 36/12 Item Short-form healthy survey; PDI-I – Patient dignity inventory; WPI – Widespread pain index; WPI-SS – Widespread pain index severity scale; FOSQ Functional outcome of sleep questionnaire; TSS – Tiredness symptoms scale; SRSBQ – Sleep related and safety behavior questionnaire; RSQ-D – Daily restorative sleep questionnaire; TP – Tender Points; HAQ – Health assessment questionnaire; MPI – multidimensional poverty index; QOL – Quality of life; BSQ –Bodyshape questionnaire; FFQ – Food frequency questionnaire; Prot – protein; CH – Carbohydrates; NCGS – nonceliac gluten sensitivity; BMI – Body mass index; GI – gastrointestinal; IL-6 interleukin-6; CRP – C-reactive protein; ESR – Erythrocyte sedimentation rate

Table 1. Dietary interventions in fibromyalgia (Cont.)

Reference	Study Design and Participants	Intervention	Outcome Measures	Results
[54]	RCT Diet Group: n=43 FM obese patients (37 female) Age: 44.8 ± 13.6 Y Control Group: n=43 FM obese patients (38 female) Age: 46.3 ± 14.4 Y	Hypocaloric diet (1200kcal/d: 20% Prot; 50% CH; 30% Fat) (G1) VS isocaloric diet (G2) 6 months	- Anthropometric data: weight, BMI, waist perimeter - Questionnaires: FIQ, TP, BDI, PSQI - Biomarkers: IL6 and CRP	<u>Comparison before and after the intervention:</u> - ↓ pain associated with FM ($p < 0.05$), localized pain ($p < 0.001$), fatigue ($p < 0.05$) and depression ($p < 0.001$) in G1 - ↓ IL6 and CRP ($p < 0.05$) in G1 <u>Comparison between groups:</u> - ↓ IL6 ($p = 0.034$) and CRP ($p = 0.07$) in G1 vs G2
[53]	UCT Female FM patients (n=48) Age: 54.5 ± 8.1 Y	Hypocaloric diet (weight loss program with weekly group sessions) 5 months (31 women completed the intervention)	- Anthropometric data: weight, BMI, waist circumference - Questionnaires: FIQ, HAQ, MPI, BDI, STAI, QOL, BSQ - Food Diary (5 days)	<u>Comparison before and after the intervention:</u> - ↓ pain associated with FM ($P = 0.00$), severity ($p = 0.04$) and day-to-day pain interference ($p < 0.001$) - ↑ body image ($p < 0.001$) and quality of life ($p < 0.001$) - ↓ anxiety ($p < 0.001$) and depression ($p < 0.001$) <u>Correlation between variables:</u> - Positive correlation between ↓ BMI and ↓ FM-associated pain ($p = 0.02$) and day-to-day pain interference ($p < 0.001$)

RCT – Randomized controlled trial; UCT – Unrandomized controlled trial; FIQR – Revised Fibromyalgia impact questionnaire; FSQ – Functional status questionnaire; IBS-SSS – Irritable bowel syndrome – severity scoring system; EQ-5D – Five dimension Euro quality of life; VAS – Visual analogue pain scale; BPI - Brief ain inventory; PSQI - Pittsburg sleep quality index; BDI – Beck's depression inventory; STAI – State trait anxiety inventory; SF36/12 – 36/12 Item Short-form healthy survey; PDI-I – Patient dignity inventory; WPI – Widespread pain index; WPI-SS – Widespread pain index severity scale; FOSQ Functional outcome of sleep questionnaire; TSS – Tiredness symptoms scale; SRSBQ – Sleep related and safety behavior questionnaire; RSQ-D – Daily restorative sleep questionnaire; TP – Tender Points; HAQ – Health assessment questionnaire; MPI – multidimensional poverty index; QOL – Quality of life; BSQ –Bodyshape questionnaire; FFQ – Food frequency questionnaire; Prot – protein; CH – Carbohydrates; NCGS – nonceliac gluten sensitivity; BMI – Body mass index; GI – gastrointestinal; IL-6 interleukin-6; CRP – C-reactive protein; ESR – Erythrocyte sedimentation rate

Table 1. Dietary interventions in fibromyalgia (Cont.)

Reference	Study Design and Participants	Intervention	Outcome Measures	Results
[55]	CCT Diet Group n=18 FM female patients Age: 51 Y Control Group n=15 FM female patients Age: 52 Y	Vegan Diet (raw veg, fruit, whole grains, oilseeds and legumes) (VD) VS Omnivorous Diet (OD) 3 months	- Questionnaires: TP, VAS pain, BDI, HAQ - Biomarkers: Hematocrit, ESR, total cholesterol, urinary sodium - Food diary (5 days)	<u>Comparison before and after the intervention:</u> - ↓ pain ($p < 0.005$) but not significant of the PT ($p = 0.07$) - ↑ autonomy ($p = 0.03$), sleep quality ($p = 0.01$), morning stiffness ($p = 0.0001$) - ↓ total cholesterol ($p < 0.003$) and urinary Na ($p = 0.0001$) - no significant statistics differences on depression, ESR and hematocrit
[59]	UCT FM patients (n=30), (28 female) Age:	Raw vegan diet (raw veg, fruit, whole grains, oilseeds) 7 months (20 adults completed the intervention)	- Questionnaires: FIQR, SF36, QOL, FFQ	<u>Comparison before and after the intervention:</u> - ↓ pain associated with FM ($p < 0.05$) - ↑ vitality, mobility, emotional health and general well-being after 7 months ($p < 0.01$) - ↑ general quality of life ($p < 0.05$)
[60]	RCT Diet Group n=36 FM female patients Age: 42.3 ± 8.4 Y Control Group n=36 FM female patients Age: 39.6 ± 8.2 Y	Diet free of monosodium glutamate (G1) VS diet without dietary restrictions (G2, on waiting list) 3 months	- Questionnaire: VAS pain - Food diary (3 months)	<u>Comparison between groups, before and after the intervention:</u> - no significant statistical differences

RCT – Randomized controlled trial; UCT – Unrandomized controlled trial; FIQR – Revised Fibromyalgia impact questionnaire; FSQ – Functional status questionnaire; IBS-SSS – Irritable bowel syndrome – severity scoring system; EQ-5D – Five dimension Euro quality of life; VAS – Visual analogue pain scale; BPI - Brief ain inventory; PSQI - Pittsburg sleep quality index; BDI – Beck's depression inventory; STAI – State trait anxiety inventory; SF36/12 – 36/12 Item Short-form healthy survey; PDI-I – Patient dignity inventory; WPI – Widespread pain index; WPI-SS – Widespread pain index severity scale; FOSQ Functional outcome of sleep questionnaire; TSS – Tiredness symptoms scale; SRSBQ – Sleep related and safety behavior questionnaire; RSQ-D – Daily restorative sleep questionnaire; TP – Tender Points; HAQ – Health assessment questionnaire; MPI – multidimensional poverty index; QOL – Quality of life; BSQ –Bodyshape questionnaire; FFQ – Food frequency questionnaire; Prot – protein; CH – Carbohydrates; NCGS – nonceliac gluten sensitivity; BMI – Body mass index; GI – gastrointestinal; IL-6 interleukin-6; CRP – C-reactive protein; ESR – Erythrocyte sedimentation rate.

The included studies presented distinct dietary interventions: low FODMAPs diet [56]; gluten-free diet [57]; a Khorosan-wheat based replacement diet [58]; monosodium glutamate- and aspartame-free diet [60]; hipocaloric diet [53, 54]; and raw vegetarian diet [55, 59]. According to the results of the Systematic Review [52], a hypocaloric diet, a raw vegetarian diet or a low FODMAPs diet may improve pain and functional repercussion in FM patients. Moreover, the decrease in GI symptoms associated with a low FODMAPs diet intervention was related with a decrease in pain and functional repercussion [56], revealing a possible association of these symptoms and intestinal microbiota changes. In parallel, high body mass index has been directly and significantly correlated to pain and functional repercussion in FM patients [53], suggesting that obesity could influence the symptoms of the disease. Other authors have postulate that fact previously [61], since adipocytes produce pro-inflammatory cytokines that could prorogate the pain. Furthermore, some studies pointed the existence of an association between FM and intestinal inflammation [2, 24-26], which suggests that in addition to weight reduction, a diet with an anti-inflammatory potential could contribute to improve disease symptoms.

In this sense, the previously mentioned association between FM and intestinal inflammation [2, 24-26] suggests that potentially inflammatory foods may play a crucial role in aggravating systemic inflammation [62] and, consequently, in the evaluation parameters of the disease. The literature suggests that saturated fatty acids, *trans* fatty acids and cholesterol, included in the "Dietary Inflammatory Index" [63, 64], together with gluten [65], dairy products [66] and ultra-processed foods [67, 68], could have a pro-inflammatory effect. Additionally, it is known the anti-inflammatory potential of mono- and poly-unsaturated fatty acids (PUFA) [64], specially Omega 3 [69], and some antioxidants compounds [70].

However, the dietary interventions presented in Table 1 used different methods to evaluate the effect of the intervention in Patient Reported Outcomes (PRO) and biomarkers parameters. Additionally, the divergence in methodology and follow-up time for each intervention, increases the probability of obtaining different effects on the measured outcomes. These facts further contribute to inconsistent results, which may hamper a conclusion based on a summary measurement of the various studies.

Taking into account the evidence described, and since nutritional therapy has been shown to be an important factor in improving the quality of life of FM patients [62], it seems pertinent to test

the hypothesis that a diet with anti-inflammatory properties and allowing an optimization of the intestinal microbiota may reduce intestinal inflammation and dysbiosis.

2. Objetives

2. Objetives

This study aimed to analyse the effects of a nutritional intervention poor on potentially inflammatory components low in foods rich in FODMAPs in the Patient Reported Outcomes and inflammatory biomarkers with FM.

The specific objectives of this study were:

1) To analyse the effect of an anti-inflammatory diet low in foods rich in FODMAPs Patient Reported Outcomes and inflammatory biomarkers after 3 months of intervention;
2) To compare the effect of an anti-inflammatory diet low in foods rich in FODMAPs with the WHO general recommendations for healthy eating, on Patient Reported Outcomes and inflammatory biomarkers after 3 months of intervention.

3. Methods

3. Methods

The detailed study protocol of this Randomized Controlled Clinical Trial (RCT) has been published [71] and registered in Clinicaltrials.gov with the identification number: NCT04007705.

3.1. Ethical considerations

This study was approved by the Ethics Committee of Portuguese Institute of Rheumatology, with reference number 4/2020, and was carried out in accordance with the Declaration of Helsinki (Declaration of 1975, revised in 2000). An informed consent was given to all participants, after oral and written information about the study. Each participant was given a code and the anonymity and confidentiality of the data collected was ensured.

3.2. Study design

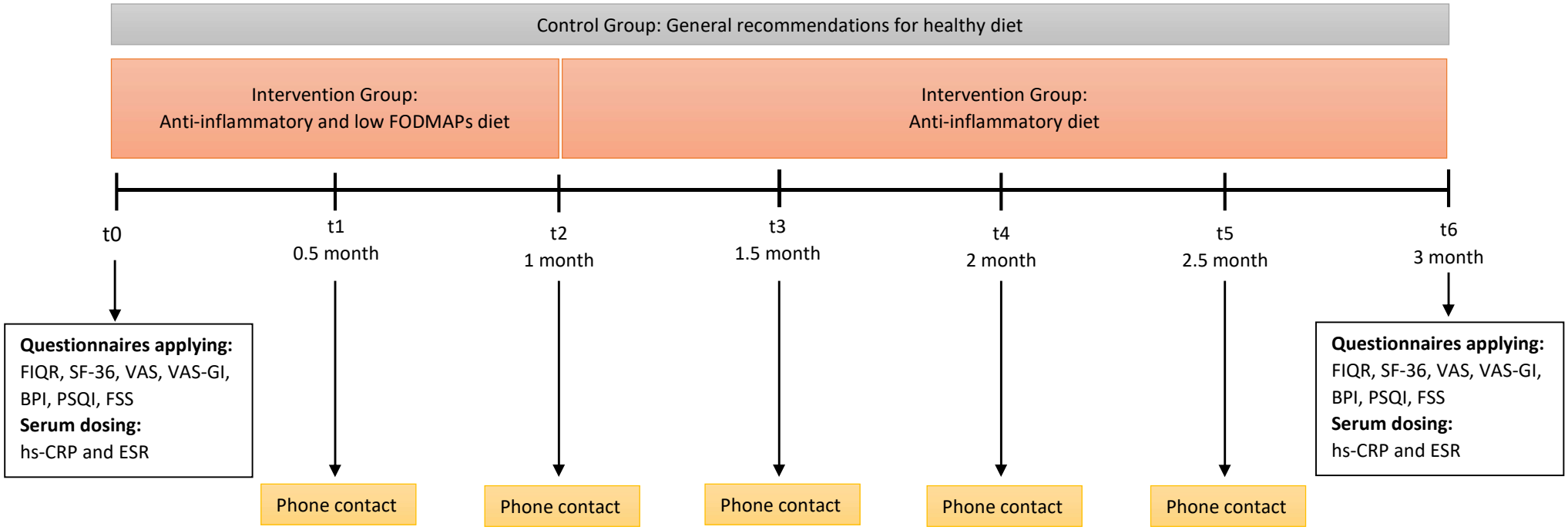
This RCT, blind to patients, took place between April 2019 and June 2020 at the Portuguese Institute of Rheumatology (*Instituto Português de Reumatologia*) in Lisbon, Portugal.

Forty-six female adults were eligible to integrate the study. For 3 months, intervention group adopted a two phases intervention: the first phase, occurred in the first month, in which an anti-inflammatory diet and low FODMAPS diet was adopted; the second phase occurred in the second and third subsequent months, and participants continued only with the anti-inflammatory diet. Control group adopted for three months a healthy diet, based on the WHO general recommendations [29].

Patients' reported outcomes (PRO) were collected in both groups by interview using structured validated questionnaires, and a blood sample was taken for the measurement of serum inflammatory biomarkers, before and after intervention. Patients were monitored through biweekly telephone contacts, being also possible for the patient to clarify any question through the contact provided.

The experimental design of the present study is shown schematically in Figure 1.

Figure 1. Experimental design of the randomized controlled clinical trial



FIQR – Revised fibromyalgia impact questionnaire; VAS – Visual analogue pain scale; VAS-GI - Visual analogue pain scale of gastrointestinal symptoms; BPI - Brief pain inventory; PSQI - Pittsburg sleep quality index; SF36 – Short-form healthy survey 36; hs-CRP – high sensitive C-Reactive protein; ESR – Erythrocyte sedimentation rate

3.3. Participants and eligibility criteria

Patients with a diagnosis of fibromyalgia followed at the Portuguese Institute of Rheumatology were invited to participate in this study, after considering the exclusion criteria (n=62). Of these, 61 agreed to participate in the study and were randomized into the 2 groups (intervention and control).

The following inclusion criteria were considered:

1- Female adults, aged over 18 and under 75 years old;

2- Diagnosis of FM performed by the rheumatology doctor, according to the Rome III criteria of the American College of Rheumatology, revised in 2010 [5];

3- Ability to read and sign the Informed Consent;

4- Stable dose therapy within 4 weeks before the study begins.

The following exclusion criteria were considered:

1- Patients with pathologies that prevent to follow the dietary intervention;

2- Patients currently undergoing lactation or pregnancy;

3- Prior or current clinical history of abuse of drug or other substances;

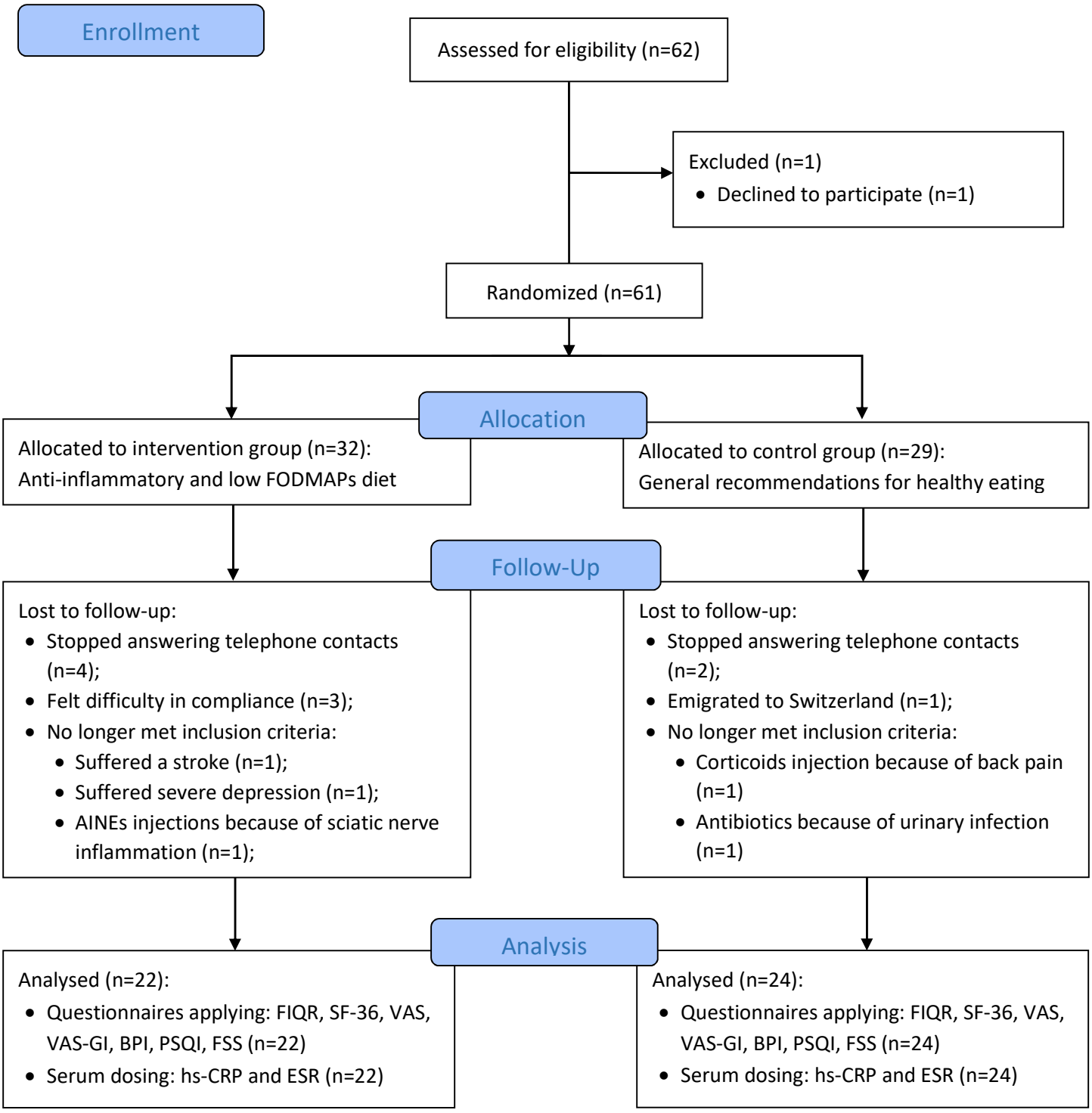
4- Change of therapy during the intervention period;

5- Presence of other inflammatory diseases;

6- Uncontrolled medical conditions (eg. Diabetes Mellitus, heart disease, renal failure, neoplastic diseases, liver diseases).

From 61 patients included initially, 46 completed the study. Reasons for lost to follow-up were diverse and are explicated in Figure 2. In the end, intervention group had 22 patients and control group had 24 patients. At baseline, the participants' mean age was 57 years.

Figure 2. CONSORT diagram of the study.



FIQR – Revised fibromyalgia impact questionnaire; VAS – Visual analogue pain scale; VAS-GI - Visual analogue pain scale of gastrointestinal symptoms; BPI - Brief pain inventory; PSQI - Pittsburg sleep quality index; SF36 – Short-form healthy survey 36; hs-CRP – high sensitive C-Reactive protein; ESR – Erythrocyte sedimentation rate

3.4. Dietary implementation

3.4.1. Intervention group

Intervention group adopted an anti-inflammatory diet, excluding potential inflammatory components/foods, such as gluten, dairy, free sugars, and ultra-processed food. Furthermore, the ingestion of foods rich in omega-3 fatty acids and flavonoids was promoted, according the "Dietary Inflammatory Index" [63, 64]. During the first month of intervention, a low FODMAPs diet criteria has been added to the anti-inflammatory diet, with the exclusion of foods rich in sugars more fermentable by bacteria. After the first month of intervention, all fruit and vegetables previously excluded were reintroduced, keeping the anti-inflammatory diet for the subsequent two months, completing a total of three months of intervention. Examples of recipes were delivered to help patients to comply with the outlined dietary plan. A table of foods to consume and to avoid was provided to participants belonging to the intervention group during low FODMAPs diet phase.

3.4.1.1. Anti-inflammatory diet

Taking into account the scientific evidence on the etiopathogenesis of FM and the potential physiological effects of foods/food components on inflammation and intestinal microbiota, we sought to create a protocol that excluded all potentially pro-inflammatory components, such as gluten, dairy, added sugar and ultra-processed foods, and included the potentially anti-inflammatory ones, such as Omega 3 fatty acids and antioxidants.

Gluten

Some authors describe an association between the characteristic symptoms of FM and the presence of altered intestinal permeability and dysbiosis [37, 39, 72]. In the presence of dysbiosis the destruction of thight juctions, proteins present in enterocytes responsible for preventing the entry of pathogens, occurs. The consequent intestinal hyperpermeability triggers, in turn, an immunological reaction of inflammatory character [23], described by several authors as intestinal low-grade inflammation [45]. Intestinal hyperpermeability appears to be caused by several

factors, including gliadin present in gluten [73-75]. Thus, it may be hypothesized that the exclusion of gluten may decrease the occurrence of dysbiosis, and therefore may decline intestinal inflammation.

Dairy

There are several different casein subtypes in milk. In bovine milk, the predominant subtype is α-casein (50-55%), which does not exist in human milk [76], besides β-casein (35%) and κ-casein (15%). In addition, there are two types of β-casein, namely A1 and A2, being the A1 β-casein the most prevalent in European dairy products [77]. A systematic review concluded that A1 β-casein was associated to a higher prevalence of GI symptoms and increased intestinal inflammation in humans, compared to A2 [78]. The mechanism seems to be related to the activation of the Th2 signaling pathway in the intestine [79], which promotes inflammation.

Another recent systematic review concluded that milk does not promote inflammation in healthy individuals and subjects with metabolic abnormalities [80]. However, given the controversy underlying the potential inflammatory effect of dairy products, we considered it prudent to exclude dairy products from the intervention.

Sugar

Sugar is a recognizably inflammatory food. In recent years, WHO has been setting up standards for reducing its ingestion to 5% of total energy intake (TEI) [81]. Its excessive consumption promotes the production of reactive oxygen species (ROS), leading to an increase in oxidative stress [82]. Additionally, a hyperinsulinogenic environment enhances the expression of pro-inflammatory molecules [83].

Ultra-processed foods

Several authors define ultra-processed food as potentially inflammatory, mainly due to its free sugars, hydrogenated fat and food additives content [84, 85]. Additionally, it is known that its relevant accumulation of advanced glycation end products (AGEs) is also related to a pro-inflammatory effect [86, 87]. When ingested, AGEs cross the epithelial barrier, attaching to the

receptors in the dendritic cells of the mucosa, and promote the uptake of the antigens and to T cells, specifically Th1, Treg, Th2 and Th17, pro-inflammatory and inducers of allergic process [88]. AGEs in the cell activate cascades of signaling the production of inflammatory molecules, such as TNF-α, IL6 and vascular cell adhesion molecule 1 (VCAM-1) [89].

Anti-inflammatory food components

To increase antioxidant and anti-inflammatory potential, the ingestion of three pieces of fruit a day and half a plate of vegetables twice a day was promoted. The intake of berries, strawberries, pomegranates, red grapes, apple (rich in flavanols, such as resveratrol and quercetin), orange, kiwi and papaya (rich in vitamin C) was indicated. Also, it was promoted the intake of broccoli, cauliflower and cabbage (rich in indole-3-carbinol and sulforaphanes), along with carrots, pumpkins, orange sweet potatoes (beta-carotene rich foods), tomato (rich in lycopene), ginger (rich in gingerol), and green tea and cocoa (rich in catechins) [90]. Antioxidants in foods are known to decrease ROS production, which in turn helps to decrease the oxidative stress and, consequently, the expression of pro-inflammatory molecules [70, 91].

Moreover, it is well known the omega-3 anti-inflammatory capacity, especially at an adequate omega-6:omega-3 ratio. It allows the production of prostaglandins, leukotrienes, resolvins and protectins, promoting the expression of anti-inflammatory cytokines [69]. Therefore, the consumption of omega-3 rich food such as salmon, mackerel, sardines and tuna, as well as walnuts, almonds and linseeds, was promoted. Furthermore, the replacement of sunflower oil, butter and margarines for extra virgin olive oil was also indicated, for an increase of monounsaturated fatty acids and reduction in omega-6 and saturated fat. Additionally, the maintenance of glycemic index was promoted, through an adequate intake of dietary fiber, protein and fat, and a balanced intake of carbohydrates, since is one of the most important factors in an anti-inflammatory diet.

3.4.1.2. Low FODMAPs diet

The presence of dysbiosis [26, 43, 72], and in particular of SIBO [20, 22], has been described in FM patients. It was observed a significant improvement in pain fatigue, gastric pain, mobility and

gastrointestinal symptoms after 4 weeks of low FODMAPs diet [56]. Marsh and colleagues meta-analysis support the efficacy of a diet with a low intake of foods rich in FODMAPs for a period of 4 to 6 weeks in the treatment of gastrointestinal symptoms, including abdominal pain, abdominal distention, constipation, diarrhea and flatulence [20].

The low FODMAPs diet is characterized by the avoidance of all dairies; all cereals except rice and oat; cashew; all fruits other than banana, citrus, pineapple, red berries, strawberries and kiwi; and all vegetables other than pumpkin, cabbage, lettuce, tomato, carrot and cucumber.

Participants in the intervention group received a table of foods to avoid (high FODMAPs) and to prefer (low FODMAPs), as well as recipes to facilitate adherence to the diet.

3.4.2. Control group

The control group was advised to adopt the general healthy eating WHO recommendations which were explained to participants. According to WHO, a healthy diet contains at least 400g of fruits and vegetables, excluding potatoes, sweet potatoes, cassava and starchy roots. A consumption of legumes, nuts and whole grains (wheat, maize, millet, oats, rice, rye), was also promoted, as well as an intake of less than 5 g of salt per day, less than 10% of total energy intake from free sugars and less than 30% of total energy intake from fats, giving preference to unsaturated fats [92].

3.5. Patient reported outcomes

The primary PRO of interest for this study were pain, fatigue, quality of sleep, quality of life and gastrointestinal symptoms, which were assessed through specific questionnaires.

Revised Fibromyalgia Impact Questionnaire (FIQR) [93], was used to assess the impact of FM on the patient's life. It consists of 21 questions that evaluate clinical severity, health status and ability to daily activities of FM patients. A score between 0 and 100 is obtained, which is lower as the quality of life improves.

Visual Analogue Pain Scale (VAS) [94] and Brief Pain Inventory (BPI) were used to assess pain [95]. VAS is a one item questionnaire about pain, which score range is between 0 (no pain)

and 10 (the worst pain ever felt). BPI measures pain intensity and pain interference in daily activities. The score ranges between 0 and 20, being lower as lower pain is felt.

To assess gastrointestinal symptoms, Visual Analog Scale from a list of common gastrointestinal and extraintestinal symptoms in FM, IBS and Non-Celiac Gluten Sensitivity (VAS_GI) [96, 97] was applied. VS_GI score was between 0 and 10, being 0 equivalent to very good gastrointestinal function and 10 very bad gastrointestinal function.

Fatigue Severity Survey (FSS) [98] was used to assess the fatigue level. This tool is a 9 items questionnaire which evaluates motor aspects of fatigue and its impact on individual's daily functioning. The scale ranges from 0 to 7 and reveals less fatigue the lower the score obtained.

Pittsburg Sleep Quality Index (PSQI) [99] was used to assess the quality of sleep. This questionnaire evaluates subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, use of sleeping pills and daytime dysfunction. PSQI score range is between 0 and 21. A total score above 5 indicates poor sleep quality.

To assess quality of life, Short-form Health Survey 36 (SF-36) [100, 101] was used. SF-36 is a 36 items tool that focus general health, physical functioning, vitality, physical pain, mental health, social functioning, and emotional impact on daily tasks. Score range is between 0 and 100, being 100 equivalent to the better possible quality of life. It encompasses both Mental and Physical Health that were quantified separately, in addition to the whole questionnaire.

3.6. Biochemical parameters assessment

A blood sample was collected at baseline and post-intervention (after 3 months). Blood tests were carried out by analysts from *Joaquim Chaves Saúde* Laboratory, at Portuguese Institute of Rheumatology. Serum high-sensitive CRP (hs-CRP) and Erythrocyte Sedimentation Rate (ESR) were measured through immunoturbidimetry [102] and Westergren method [103] respectively, to assess the presence of inflammation. Despite being both nonspecific markers, the combination of both allows obtaining information on the individual's inflammatory phenotype. Being an acute phase protein, CRP reveals the presence of inflammation in its initial phase, increasing after 4-6 hours. On its turn, the ESR increases within 24 to 48h and gradually decreases, allowing to assess

the response to a treatment [104]. Additionally, high levels of serum CRP in FM correlates to ERS, IL-8 and IL-6 [105].

3.7. Socio-demographic and life-style characteristics assessment

Socio-demographic characteristics of the patients were collected, namely age, education level (further grouped in < 9 schooling years or ≥ 9 schooling years) and work status (employed, unemployed, retired or domestic/pensioner).

Life-style characteristics, such as smoking habits (recoded as smoker or non-smoker), frequency of alcohol beverages intake (recoded as daily drinkers; or occasionally and non-drinkers, since only one participant reported a regular consumption) and structured physical exercise (further grouped in < 1 hour a week or ≥ 1 hour a week), were collected. Additionally, it was also registered the disease duration since diagnosis and usual pharmacological therapy.

3.8. Anthropometric and body composition assessment

Data on anthropometric measurements namely waist circumference, height and weight were assessed at beginning and in the end of the intervention, through the scale Inbody ®, model 770. Body mass index (BMI) (kg/m²) was calculated, and WHO classification was used to categorize BMI [106].

Body composition parameters namely fat mass percentage, muscular mass and total body water were estimated by bio-impedance, through the scale Inbody ®, model 770.

3.9. Dietary and nutritional assessment

At baseline, a 24-hour dietary recall was applied to verify the homogeneity on dietary intake between groups (intervention and control). Every biweekly telephone contact and at the end of the intervention, a 3-day food record was completed by each participant in order to assess the intervention compliance. Study participants were carefully instructed by a dietitian/nutritionist to record a food diary of the 72-hours prior to each phone contact.

The Food Processor ® software version 11.2.274 was used to convert food into nutrients. Energy and nutrients were expressed by average values calculated from the 3-day food records. Protein, carbohydrates, of which sugars, monosaccharides, disaccharides and added sugars, total fat, of which monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), omega-3 and omega-6 were expressed by percentage of TEI (% TEI). Dietary fiber was expressed in grams and g/1000kcal.

Additionally, the average of the 3-day food record of the ingested amount of food containing gluten in its composition (bread, biscuits, cake, pasta, breakfast cereals, cereal bars) was manually collected from food diaries and 24 hours food recalls. The same foods in the gluten-free version were not considered. Moreover, dairy products (milk, yogurt, cheese, butter), ultra-processed products classified according to the NOVA classification system [107], and sugar added to beverages were also collected and expressed in grams.

3.10. Data analysis

Descriptive data were presented as mean, standard deviation (SD), median, percentile (P) 25 and P75 for continuous variables or the frequency (number and percentage) for categorical variables. Post-intervention and baseline difference was arithmetically calculated for anthropometric and body composition, dietary intake, PRO and biochemical parameters variables.

To compare FM symptoms and inflammatory biomarkers within-groups at baseline and post-intervention, Paired Samples T-Test or Wilcoxon signed rank test were used for continuous variables, as appropriate.

Independent Samples T-Test or Mann-Whitney U test were used to compare FM symptoms, inflammatory biomarkers and dietary intake between groups at baseline and at post-intervention moments, as appropriate. The arithmetic differences between baseline and post-intervention were calculated for dietary intake and PRO for each group. MANOVA was applied to assess the effect of the intervention between groups.

Additionally, a General Linear Model (GLM) was used in order to assess the impact of the intervention adjusting for potentially confounders, namely age, disease duration, variation of

BMI and variation of body fat percentage. GLM was also used to verify the possible isolated effect of each nutrient and food with anti-inflammatory potential in the PRO.

Statistical analysis was performed using IBM SPSS Statistics Software, version 19.0.

In order to define the sample size required for the study and to give a statistical power of 80%, G-Power Software version 3.1.9.4 revealed that, for a desirable effect size of 50%, a minimum sample size of 45 individuals was required.

4. Results

4. Results

Here we present the findings of our investigation, in respect to participants baseline characteristics, dietary nutritional data, and the effect of the anti-inflammatory and low FODMAPs diet in patient reported outcomes.

4.1. Baseline characteristics of the participants

The study sample consisted of 62 adult female FM patients of which 46 patients completed the study. There were no significant differences between intervention group (n = 22) and control group (n = 24) for demographics, life-style characteristics and body composition (Table 2).

Almost 40% of the participants were employed and had less than 9 schooling years. More than 85% reported being non-smoker, more than 91% did not drink alcoholic beverages daily and more than 91% exercised less than 1 hour a week. Both groups had a body fat mass average of 39%, and BMI of nearly 30 kg/m^2.

Regarding usual pharmacological treatment, over than 50% in both groups were medicated with analgesics and muscle relaxants, and approximately 75% reported to take antidepressants, anxiolytics, or sedatives.

Table 2. Baseline socio-demographic and lifestyle characteristics of the participants

Characteristics	Control Group (*n*= 24) Mean (±SD) Median (P25;P75)	Intervention Group (*n*= 22) Mean (±SD) Median (P25;P75)	*p*-value
Age (years)	56 (±8) 57 (51; 59)	60 (±6) 60 (56; 66)	0.057[a]
Disease duration (years)	13 (±9) 13 (4; 20)	14 (±8) 17 (5; 20)	0.526[a]
Body mass and composition			
Body mass index (kg/m^2)	30 (±6) 29 (26; 34)	29 (±4) 29 (25; 31)	0.531[a]
Waist circumference (cm)	99 (±14) 101 (90; 109)	98 (±10) 101 (89; 106)	0.783[a]
Fat mass (%)	39 (±9) 41 (33; 44)	39 (±6) 38 (34; 44)	0.796[a]
Muscle mass (kg)	24 (±3) 24 (21; 27)	23 (±2) 23 (21; 25)	0.502[b]
Total body water (%)	45 (±7) 43 (41; 50)	45 (±6) 45 (41; 47)	0.758[b]
	***n* (%)**	***n* (%)**	
Education (schooling)			
< 9 years	14 (60.9)	10 (45.5)	0.388
≥9 years	9 (39.1)	12 (54.5)	0.152
Work status			
Employed	10 (43.5)	8 (36.4)	0.541
Unemployed	3 (13.0)	1 (4.5)	0.344
Retired	5 (21.7)	8 (36.4)	0.248
Domestic / pensioner	5 (21.7)	5 (22.7)	0.327
Smoking habits			
Smoker	2 (8.7)	3 (13.6)	0.568
Nonsmoker	21 (91.3)	19 (86.4)	0.777
Alcoholic beverages consumption			
Daily	2 (8.7)	0 (0)	0.338
Occasional/Never	21 (91.3)	22 (100)	0.090
Exercise frequency			
<1 hour/week	22 (91.7)	18 (81.8)	0.596
≥1 hour/week	2 (8.3)	4 (18.2)	0.823

SD, standard deviation; P25, percentile 25; P75, percentile 75.

[a]*p*-value calculated by Independent-Samples T-Test between control and intervention groups mean values;

[b]*p*-value calculated by Mann-Whitney Test between control and intervention groups mean values.

4.2. Dietary and nutritional data

At baseline, no significant differences were observed between groups in most of the nutritional parameters, except for the intake of total energy and omega-3 fatty acids, and for the consumption of added sugars and ultra-processed products which were significantly higher in control group (Table 3).

The control group maintained dietary intake, with no differences between baseline and post intervention. However, intervention group reported significant changes after the implementation of the dietary protocol, with a negative variation in the contribution to TEI for protein (-2.1 ± 4.2 % to TEI, p=0.03), carbohydrates (-5.9 ± 9.9 % to TEI, p=0.011), sugars (-7.5 ± 9.1 % to TEI, p=0.001), disaccharides (-3.3 ± 3.0 % to TEI, p<0.001) and SFA (-3.0 ± 4.1 % to TEI, p=0.006). On the contrary, a positive variation was found for total fat (9.4 ± 9.8 % to TEI, p=0.001), PUFA (5.0 ± 10.1 % to TEI, p=0.022), omega-3 fatty acids (0.7 ± 0.046), and fibre/1000kcal (0.4 ± 0.9 % to TEI, p=0.037). Additionally, intervention group reported the exclusion of sugar added to foods (baseline 1.1 ± 3.7 g; post-intervention 0 g, p<0.001) and ultra-processed foods (baseline 47.3 ± 44.1 g; post-intervention 0 g, p<0.001), as prescribed.

Despite the statistically similar baseline values, there were significant differences between intervention and control group in the post-intervention period regarding the intake of disaccharides, added sugar and SFA, which was higher in control group, and concerning the intake of total fat and PUFA that was higher in intervention group (Table 3).

Table 3. Dietary intake in control and intervention group at baseline[1] and post-intervention[2].

Outcomes	Control Group (*n* = 24)				Intervention Group (*n* = 22)				Between-group analysis	
	Baseline Mean (± SD) Median (P25;P75)	**Post-intervention Mean (± SD) Median (P25;P75)**	**Post-intervention – Baseline Difference Δ Mean (± SD) Δ Median (P25; P75)**	**Within-group analysis *p*-value**	**Baseline Mean (± SD) Median (P25;P75)**	**Post-intervention Mean (± SD) Median (P25;P75)**	**Post-intervention – Baseline Difference Δ Mean (± SD) Δ Median (P25; P75)**	**Within-group analysis *p*-value**	**Baseline p-value**	**Post-intervention *p*-value**
Total energy intake (kcal)	1773 (±374) 1710 (1488; 2030)	1725 (±374) 1722 (1397; 1976)	-48.3 (±446.5) 68.7 (420.1; 229.4)	0.775[b]	1471 (±362) 1455 (1255; 1736)	1256 (±355) 1320 (1176; 1403)	-195.8 (±544) -13.9 (-412.3; 140.5)	0.236[b]	0.008[d]	*p*<0.001[d]
Protein (% TEI)	20 (±5) 19 (17; 23)	19 (±3) 20 (17; 22)	-1.2 (±4.9) -0.8 (-4.0; 2.2)	0.246[a]	21 (±4) 21 (19; 24)	19 (±3) 18 (17; 22)	-2.1 (±4.2) -1.6 (-4.8; 0.4)	0.030[a]	0.657[d]	0.777[c]
Carbohydrate (% TEI)	49 (±8) 50 (45; 55)	49 (±5) 50 (46; 52)	0.4 (±6.4) -0.2 (-4.2;3.9)	0.767[a]	51 (±9) 53 (44; 57)	46 (±6) 46 (41; 49)	-5.9 (±9.9) -4.9 (13.2; 1.4)	0.011[a]	0.385[d]	0.049[c]
Sugars (% TEI)	19.7 (±7.1) 20.0 (13.5; 23.8)	14.0 (±7.7) 15.2 (8.1; 19.5)	-5.7 (±8.9) -4.8 (-8.7; -0.7)	0.005[a]	19.8 (±7.6) 18.3 (14.2; 26.4)	12.3 (±7.1) 14.2 (9.2; 15.6)	-7.5 (±9.1) -7.6 (-14.6; -1.3)	0.001[a]	0.981[d]	0.416[c]
Monossacharides (% TEI)	5.0 (±2.3) 4.7 (2.9; 7.2)	5.2 (±2.9) 4.9 (3.2; 6.9)	0.2 (±3.3) 0.4 (-2.4; 2.9)	0.821[a]	4.9 (±2.9) 4.6 (2.6; 6.3)	5.5 (±3.4) 5.8 (3.6; 7.6)	-0.5 (±4.1) 0.9 (-2.4; 2.7)	0.542[a]	0.885[d]	0.778[c]
Dissacharides (% TEI)	4.6 (±3.0) 4.2 (2.1; 7.0)	4.2 (±2.5) 3.9 (2.6; 5.6)	0.4 (±2.6) 0.2 (-1.6; 1.8)	0.440[a]	4.9 (±2.6) 5.2 (2.6; 6.6)	1.7 (±1.3) 1.5 (0.9; 2.7)	-3.3 (±3.0) -3.5 (5.1; 0.9)	*p*<0.001[a]	0.737[d]	0.001[c]
Added sugars (% TEI)	0.8 (±1.6) 0.0 (0.0; 1.7)	0.7 (±0.9) 0.0 (0.0; 1.4)	-0.1 (±1.3) 0.0 (0.0; 0.3)	0.386[b]	0.5 (±1.4) 0.0 (0.0; 0.0)	0.0 (±0.0) 0.0 (0.0; 0.0)	-0.5 (±1.5) 0.0 (0.0; 0.0)	0.144[b]	0.186[c]	0.003[d]
Dietary fiber (g)	17.9 (±3.7) 17.7 (14.9; 20.3)	17.0 (±7.6) 18.3 (11.5; 21.7)	-0.9 (±8.1) 0.9 (-6.6; 3.6)	0.710[b]	16.0 (±5.6) 16.4 (10.7; 19.9)	16.5 (±9.5) 17.5 (13.6; 21.6)	0.5 (±11.5) 0.9 (-6.8; 7.3)	0.858[b]	0.235[c]	0.930[d]
Dietary fiber (g/1000 kcal)	1.7 (±0.4) 1.8 (1.5; 2.0)	1.9 (±0.5) 1.8 (1.3; 2.2)	0.1 (±0.6) 0.2 (-0.4; 0.4)	0.680[a]	1.6 (±0.6) 1.6 (1.0; 1.9)	2.0 (±0.6) 1.8 (1.6; 2.5)	0.4 (±0.9) 0.4 (-0.3; 0.8)	0.037[a]	0.169[d]	0.343[c]

[1]Values refer to 24h prior first contact (at baseline).

[2]Values are the average of the 3 days prior to the date of post intervention.

[3]Amount of food containing gluten in its composition (bread, biscuits, cake, pasta, savoury, breakfast cereals, cereal bars)

SFA = Saturated Fatty Acid; MUFA = Monounsaturated fatty acid; PUFA = Polyunsaturated fatty acid; n-3 = Omega 3 Fatty Acid; n-6 = Omega 6 Fatty Acid; TEI = Total Energy Intake.

[a]*p*-value calculated by Paired Samples T-Test between baseline and post-intervention, within-groups mean values;

[b]*p*-value calculated by Wilcoxon Test between baseline and post-intervention, within-groups mean values;

[c] *p*-value calculated by Independent-Samples T-Test between control and intervention groups mean values;
[d] *p*-value calculated by Mann-Whitney Test between control and intervention groups mean values.

Table 3. Dietary intake in control and intervention group at baseline[1] and post-intervention[2] (Cont.)

Outcomes	Control Group (*n* = 24)				Intervention Group (*n* = 22)				Between-group analysis	
	Baseline Mean (± SD) Median (P25;P75)	**Post-intervention Mean (± SD) Median (P25;P75)**	**Post-intervention – Baseline Difference Δ Mean (± SD) Δ Median (P25; P75)**	**Within-group analysis *p*-value**	**Baseline Mean (± SD) Median (P25;P75)**	**Post-intervention Mean (± SD) Median (P25;P75)**	**Post-intervention – Baseline Difference Δ Mean (± SD) Δ Median (P25; P75)**	**Within-group analysis *p*-value**	**Baseline *p*-value**	**Post-intervention *p*-value**
Total fat (% TEI)	30 (±6) 30 (26; 34)	31 (±6) 31 (27; 35)	1.2 (±7.4) -0.1 (-3.4; 7.9)	0.407[b]	28 (±8) 27 (23; 29)	37 (±7) 37 (32; 41)	9.4 (±9.8) 10.5 (0.6; 14.5)	0.001[b]	0.071[c]	0.004[c]
SFA (% TEI)	8.2 (±2.1) 8.3 (6.4; 10.4)	7.3 (±3.0) 7.5 (6.0; 10.1)	-0.9 (±2.9) 0.1 (-2.3; 0.9)	0.440[b]	7.8 (±2.5) 7.6 (6.2; 10.1)	4.9 (±2.7) 5.7 (3.6; 6.9)	-3.0 (±4.1) -2.3 (-6.6; 0.2)	0.006[b]	0.716[d]	0.004[d]
MUFA (% TEI)	5.7 (±2.5) 5.5 (4.2; 6.6)	4.8 (±2.1) 5.1 (3.9; 5.8)	-0.9 (±3.9) -0.3 (-2.1; 0.9)	0.331[b]	4.6 (±1.8) 4.6 (3.3; 5.4)	6.6 (±4.9) 5.9 (3.3; 9.0)	1.9 (±5.6) -0.8 (-1.7; 6.1)	0.123[b]	0.062[c]	0.117[c]
PUFA (% TEI)	13.0 (±4.5) 12.9 (10.2; 15.0)	13.9 (±5.2) 13.8 (11.9; 17.5)	0.8 (±7.3) 1.6 (-2.2; 5.9)	0.278[b]	11.2 (±5.2) 11.1 (7.3; 13.7)	16.2 (±8.4) 19.3 (14.4; 21.1)	5.0 (±10.1) 7.9 (1.2; 13.2)	0.022[b]	0.206[d]	0.018[d]
n-3 (% TEI)	0.9 (±0.6) 0.8 (0.5; 1.0)	0.9 (±0.5) 0.7 (0.6; 1.2)	-0.1 (±0.9) 0.1 (-0.2; 0.3)	0.530[b]	0.6 (±0.4) 0.4 (0.3; 0.6)	1.3 (±0.9) 1.3 (0.4; 1.9)	0.7 (±1.4) 0.4 (-0.4; 1.6)	0.046[b]	0.006[c]	0.538[d]
n- 6 (% TEI)	4.5 (±1.8) 4.2 (3.5; 5.1)	3.9 (±1.8) 4.1 (3.0; 4.8)	-0.7 (±2.9) -0.3 (1.6; 0.9)	0.317[b]	3.7 (±1.7) 3.6 (2.6; 4.2)	5.1 (±3.9) 4.6 (2.5; 8.2)	1.4 (±4.5) 0.9 (-1.8; 5.6)	0.149[b]	0.129[d]	0.391[d]
Food containing gluten[3] (g)	179.8 (±92.4) 187.5 (105; 260)	150.9 (±54.9) 153.3 (116.3; 185)	-28.9 (±86.9) -14.2 (66.7; 28.3)	0.118[a]	170.6 (±71.8) 162.5 (118.8; 205)	0 0	-170.6 (±71.7) -162.5 (-205.0; -118.8)	*p*<0.001[b]	0.707[c]	*p*<0.001[d]
Dairy products (g)	303.1 (±210) 234.6 (131.3; 501.3)	254.3 (±216.3) 235 (55.4; 358.7)	-48.8 (±150.9) 150.9 (-144.8; 54.2)	0.127[a]	290.2 (±220.3) 220.0 (138.3; 411.3)	0 0	-290.2 (±220.3) -220 (-411.3; -138.8)	*p*<0.001[b]	0.848[c]	*p*<0.001[d]
Ultra-processed foods (g)	82.4 (±67.5) 67.2 (26.3; 142.5)	52.5 (±47.3) 47 (5.0; 78.8)	-29.9 (±78.6) -5.2 (-99.8; 17.5)	0.075[a]	47.3 (±44.1) 47.5 (0.0; 7.5)	0 0	-47.3 (±44.1) -47.5 (-75.0; 0.0)	*p*<0.001[b]	0.044[c]	*p*<0.001[d]
Sugar added to foods (g)	4.0 (±0.0) 6.3 (0.0; 8.0)	3.0 (±4.5) 0 (0; 8)	-1.0 (±4.3) 0 (0; 0)	0.257[b]	1.1 (±3.7) 0 (0; 0)	0 0	-1.1 (±3.7) 0 (0; 0)	*p*<0.001[b]	0.038[d]	*p*<0.001[d]

[1]Values refer to 24h prior first contact (at baseline).

[2]Values are the average of the 3 days prior to the date of post intervention.

[3]Amount of food containing gluten in its composition (bread, biscuits, cake, pasta, savoury, breakfast cereals, cereal bars)

SFA = Saturated Fatty Acid; MUFA = Monounsaturated fatty acid; PUFA = Polyunsaturated fatty acid; n-3 = Omega 3 Fatty Acid; n-6 = Omega 6 Fatty Acid; TEI = Total Energy Intake.

[a]*p*-value calculated by Paired Samples T-Test between baseline and post-intervention, within-groups mean values;

[b]*p*-value calculated by Wilcoxon Test between baseline and post-intervention, within-groups mean values;
[c]*p*-value calculated by Independent-Samples T-Test between control and intervention groups mean values;
[d]*p*-value calculated by Mann-Whitney Test between control and intervention groups mean values.

4.3. Patient reported outcomes

The differences between post-intervention and baseline showed significantly more favourable outcomes for the majority of parameters in intervention group compared to control group. Significantly greater improvement was found in FM severity scale FIQR in intervention group compared to control group (-19.9 ± 18.8 vs -2.2 ± 16.1; *p*=0.001). Significantly greater improvement was found in pain in intervention group compared to control group, both in VAS (-2.3 ± 2.5 vs -0.04 ± 2.1; *p*=0.002) and BPI questionnaires (-3.8 ± 4.1 vs -1.1 ± 2.6; *p*=0.011. Significantly greater improvement was found in gastrointestinal symptoms, through VAS_GI questionnaire, in intervention group compared to control group (-2.0 ± 0.9 vs -0.9 ± 1.3; *p*=0.002). Significantly greater improvement was found in sleep quality, in PSQI questionnaire, in intervention group compared to control group (-3.5 ± 4.6 vs -1.2 ± 2.6; *p*=0.048). Significantly greater improvement was found in fatigue, through FSS questionnaire, in intervention group compared to control group (-1.1 ± 1.2 vs -0.5 ± 1.0; *p*=0.042). Significantly greater improvement was found in quality of life, evaluated through SF36, in intervention group compared to control group (10.2 ± 11.2 vs 3.6 ± 10.4; *p*=0.045), specifically in physical component (18.1 ± 20.0 vs 3.9 ± 13.5; *p*=0.008). SF36 score is higher as quality of life improves (Table 4).

At baseline, the between-group analysis showed no differences for the majority of parameters evaluated except for BPI, FSS and SF36, for which the intervention group had more favourable baseline values.

In respect to intervention group, there was observed an improvement between baseline and post-intervention in FIQR (59.3 ± 9.2 vs 39.5 ± 21.8; *p*<0.001), in VAS (7.7 ± 1.4 vs 5.4 ± 2.3; *p*=0.001), BPI (12.5 ± 2.3 vs 8.7 ± 4.7; *p*<0.001), FSS (5.5 ± 1.1 vs 4.4 ± 1.7; *p*=0.001), VAS_GI (3.4 ± 1.5 vs 1.4 ± 1.3; *p*<0.001), PSQI (15.0 ± 5.2 vs 11.6 ± 5.7; *p*=0.002), SF36 (44.0 ± 10.3 vs 54.3 ± 12.3; *p*<0.001); SF36 physical component (33.4 ± 11.4 vs 51.5 ± 18.8; *p*<0.001) and SF36 mental component (54.4 ± 23.1 vs 63.4 ± 21.4; *p*=0.023).

In control group, there was also found an improvement in VAS_GI (3.1 ± 1.4 vs 2.3 ± 1.3; *p*=0.007), FSS (6.4 ± 0.7 vs 5.9 ± 1.2; *p*=0.038) and PSQI (15.1 ± 4.0 vs 13.9 ± 4.5; *p*=0.037) at the end of intervention compared to baseline.

Inflammatory biomarkers (hs-CRP, ESR) did not significantly change in both groups (Table 5).

With regard to weight status and body composition, it was found that, in the control group, there were no differences between baseline and post-intervention (BMI: 29.5 ± 5.8 vs 29.2 ± 5.5; p=0.078; body fat percentage: 39.1 ± 8.9 vs 37.7 ± 10.9; p=0.181). However, in the intervention group there were significant changes between the two moments, both in BMI (28.6 ± 4.1 vs 27.6 ± 3.9, p>0.001) and body fat percentage (38.5 ± 6.4 vs 37.0 ± 7.0; p=0.015).

It was possible to observe that, the impact of the intervention on FM symptoms was beneficial in the intervention group regardless of age, disease duration, BMI variation and body fat mass variation between baseline and post-intervention. When the impact of the variation in the intake of each nutrient per se (monosaccharides, disaccharides, dietary fiber, omega 3 fatty acids and omega 6 fatty acids) on FM clinical features was tested, there were no significant differences between post-intervention and baseline moments.

The effect of the intervention between groups remains significant for FIQR, VAS and VAS_GI after a multivariate analysis.

Table 4. Clinical features in control and intervention group at baseline and post-intervention.

Outcomes	Control Group (*n* = 24)				Intervention Group (*n* = 22)				Between-group analysis		Between-group post-intervention – baseline difference analysis *p*-value
	Baseline Mean (± SD) Median (P25;P75)	Post-intervention Mean (± SD) Median (P25;P75)	Post-intervention - Baseline Difference Δ Mean (± SD) Δ Median (P25; P75)	Within-group analysis *p*-value	Baseline Mean (± SD) Median (P25;P75)	Post-intervention Mean (± SD) Median (P25;P75)	Post-intervention - Baseline Difference Δ Mean (± SD) Δ Median (P25; P75)	Within-group analysis *p*-value	Baseline *p*-value	Post-intervention *p*-value	
FIQR (Range: 0-100)	60.2 (±10.5) 60.5 (52.5; 68.9)	57.6 (±15.6) 61.2 (50.4; 68.4)	-2.2 (±16.1) -0.05 (9.1; 7.6)	0.515[b]	59.3(±9.2) 58.3 (53.3; 67.1)	39.5 (±21.8) 40.1 (23.8; 58.8)	-19.9 (±18.8) -15.8 (-34.2; -3.1)	*p*<0.001[b]	0.676[c]	0.004[c]	0.001[d]
VAS (Range: 0-10)	7.6 (±1.6) 8.0 (7.0; 8.8)	7.6 (±1.9) 8.0 (7.0; 9.0)	-0.04 (±2.1) 0.0 (-1.0; 1.0)	0.935[a]	7.7 (±1.4) 8.0 (7.0; 9.0)	5.4 (±2.3) 6.0 (3.8; 7.3)	-2.3 (±2.5) -2.5 (-4.3; -0.8)	0.001[a]	0.937[c]	0.001[c]	0.002[d]
VAS GI (Range: 0-10)	3.1 (±1.4) 3.0 (1.9; 4.7)	2.3 (±1.3) 2.2 (1.5; 2.6)	-0.9 (±1.3) -0.5 (-1.7; 1.7)	0.007[a]	3.4 (±1.5) 3.4 (2.2; 4.4)	1.4 (±1.3) 1.2 (0.1; 2.6)	-2.0 (±0.9) -2.1 (-2.7; -1.3)	*p*<0.001[a]	0.660[c]	0.023[c]	0.002[d]
BPI (Range: 0-20)	14.1 (±2.2) 14.4 (12.9; 15.2)	13.0 (±3.6) 13.4 (11.1; 15.5)	-1.1 (±2.7) -1.0 (-2.4; 1.1)	0.062[b]	12.5 (±2.3) 12.8 (10.8; 14.1)	8.7 (±4.7) 10.2 (4.4; 12.2)	-3.8 (±4.1) -3.2 (-5.7; -0.7)	*p*<0.001[b]	0.015[c]	0.001[c]	0.011[d]
PSQI (Range: 0-21)	15.1 (±4.0) 16.0 (12.0; 18.0)	13.9 (±4.5) 14.5 (11.0; 17.0)	-1.2 (±2.6) -1.0 (-2.8; 0.8)	0.037[b]	15.0 (±5.2) 15.0 (10.8; 19.5)	11.6 (±5.7) 9.5 (8.5; 16.3)	-3.5 (±4.6) -3.0 (-8.0; 0.8)	0.002[b]	0.808[c]	0.073[c]	0.048[d]
FSS (Range: 0-7)	6.4 (±0.7) 7.0 (6.0; 7.0)	5.9 (±1.2) 6.0 (5.0; 7.0)	-0.5 (±1.0) 0.0 (-1.0; 0.0)	0.038[a]	5.5 (±1.1) 6.0 (4.8; 6.0)	4.4 (±1.7) 5.0 (3.8; 5.3)	-1.1 (±1.2) -1.0 (-2.0; 0.0)	0.001[a]	0.003[c]	0.001[c]	0.042[c]
SF36 (Range: 0-100)	38.6 (±7.2) 38.9 (33.1; 42.7)	42.2 (±9.7) 42.2 (36.5; 47.2)	3.6 (±10.4) 2.2 (-4.9; 11.9)	0.137[a]	44.0 (±10.3) 42.6 (36.9; 53.5)	54.3 (±12.3) 58.4 (43.5; 63.6)	10.2 (±11.2) 9.0 (3.4; 15.9)	*p*<0.001[a]	0.047[c]	0.001[c]	0.045[c]
SF36 Physical Component (Range: 0-100)	30.9 (±8.2) 31.8 (22.6; 35.6)	34.8 (±14.3) 30.9 (21.7; 49.6)	3.9 (±13.5) 1.3 (-4.8; 13.6)	0.168[b]	33.4 (±11.4) 34.6 (25.0; 41.0)	51.5 (±18.8) 56.3 (36.5; 66.7)	18.1 (±20.0) 22.5 (-1.0; 36.0)	*p*<0.001[b]	0.454[c]	0.002[c]	0.008[d]
SF36 Mental Component (Range: 0-100)	38.6 (±15.8) 36.2 (26.2; 48.7)	47.2 (±19.8) 43.3 (28.9; 64.1)	8.5 (±23.1) 7.1 (-2.6; 24.7)	0.052[a]	54.4 (±23.1) 56.3 (33.7; 71.4)	63.4 (±21.4) 68.5 (51.3; 78.8)	8.9 (±21.0) 8.1 (1.9; 19.2)	0.023[a]	0.015[c]	0.016[c]	0.947[d]

FIQR – Revised Fibromyalgia Impact Questionnaire; VAS – Visual Analogue Pain Scale; VAS GI - Visual Analogue Scale from gastrointestinal symptoms; BPI - Brief Pain Inventory; PSQI - Pittsburg Sleep Quality Index; FSS - Fatigue Severity Survey; SF36 - Short Form 36.

[a]*p*-value calculated by Wilcoxon Test between baseline and post-intervention, within-groups mean values;

[b]*p*-value calculated by Pared Sample T-Test between baseline and post-intervention, within-groups mean values;
[c]*p*-value calculated by Mann-Whitney between control and intervention groups mean values;
[d]*p*-value calculated by T-Test for independent samples, between control and intervention mean values.

Table 5. Biochemical parameters assessment in control and intervention group at baseline and post-intervention.

Outcomes	Control Group (*n* = 24)				Intervention Group (*n* = 22)				Between-group analysis		Between-group post-intervention variation analysis *p*-value
	Baseline Mean (± SD) Median (P25;P75)	Post-intervention Mean (± SD) Median (P25;P75)	Post-intervention Δ Mean (± SD) Δ Median (P25; P75)	Within-group analysis *p*-value	Baseline Mean (± SD) Median (P25;P75)	Post-intervention Mean (± SD) Median (P25;P75)	Post-intervention Δ Mean (± SD) Δ Median (P25; P75)	Within-group analysis *p*-value	Baseline *p*-value	Post-intervention *p*-value	
hs-CRP (mg/dL)	0.33 (±0.32) 0.24 (0.09; 0.43)	0.36 (±0.44) 0.23 (0.09; 0.49)	0.03 (±0.29) -0.03 (-0.15; 0.09)	0.920[a]	0.32 (±0.27) 0.21 (0.11; 0.53)	0.37 (±0.34) 0.19 (0.11; 0.62)	0.04 (±0.26) -0.0 (0.08; 0.15)	0.745[a]	0.886[c]	0.750[c]	0.567[c]
ESR (mm)	10.42 (±8.20) 7.5 (5.0; 14.5)	9.88 (±8.83) 7.0 (5.0; 15.75)	-0.54 (±4.90) - 0.5 (-3.0; 2.75)	0.663[a]	11.36 (±8.29) 8.0 (5.0; 14.25)	11.64 (±11.16) 8.50 (4.0; 13.75)	0.27 (±6.69) 0.0 (-4.3; 3.25)	0.794[a]	0.650[c]	0.708[c]	0.640[c]

hs-CRP – high-sensitive C-Reactive Protein; ESR – Erythrocyte Sedimentation Rate.

[a] *p*-value calculated by Wilcoxon Test between baseline and post-intervention, within-groups mean values;

[b] *p*-value calculated by Pared Sample T-Test between baseline and post-intervention, within-groups mean values;

[c] *p*-value calculated by Mann-Whitney between control and intervention groups mean values;

[d] *p*-value calculated by T-Test for independent samples, between control and intervention mean values.

5. General discussion and conclusions

5. General discussion and conclusions

The findings of this study demonstrate an improvement in FM symptoms, namely pain, fatigue, gastrointestinal symptoms, quality of sleep and quality of life in intervention group, after an anti-inflammatory and low FODMAPs nutritional intervention, compared to control group.

In this chapter, we will discuss the results obtained in comparison with similar articles previously published, arguing the expected corresponding action mechanisms.

5.1. Overview of the outcomes

In our study, a wide variety of outcomes, assessed through validated instruments were considered, to broaden the ability to assess typical FM symptoms.

The differences between post-intervention and baseline assessments showed significantly more favourable outcomes for the majority of parameters in intervention group compared to control group. However, inflammatory biomarkers (hs-CRP, ESR) did not significantly change in both groups.

These results are aligned with other dietary interventions. In the period preceding our clinical trial, eight dietary interventions were published, namely a low FODMAPS diet [56], two hypocaloric diets [53, 54], two vegetarian diets [55, 59], a gluten free diet [57], a low monosodium glutamate and aspartame diet [60] and a Khorasan Wheat–Based Replacement diet [58]. In these studies, pain and functional repercussion, sleep quality, fatigue, quality-of-life and gastrointestinal disturbances were evaluated. However, half of these studies had no control group [53, 56, 59], or did not compared the results between groups [55].

Effect on pain and functional repercussion

At the end of the study, there was an improvement in the pain assessment parameters (through VAS and BPI questionnaires) and functional repercussion (FIQR) of patients in intervention group compared to control group.

These results were also found in studies of different dietary interventions. A vegetarian diet intervention [59] showed significant reduction in FIQR, after 7 months of intervention, in intervention group. In other vegetarian study, the authors described a significant reduction in VAS [55].

Similarly, two hypocaloric diet interventions also showed a significant reduction in pain. In one study [53], FIQ and Multidimensional Pain Inventory (MPI) scores decreased after 5 months of the intervention. In the other study [54], after 6 months of hypocaloric diet, the intervention group presented significantly improved FIQ scores compared to the control group.

Furthermore, an intervention with a low FODMAPs diet [56] reduced significantly pain associated with FM, evaluated by FIQR and Functional Status Questionnaire (FSQ). Additionally, this study showed a significant and positive correlation between FIQR and IBS - Symptom Severity Scale (IBS-SSS).

Although the unknown FM pathophysiology, it has been suggested that genetic predisposition and stressful life events may trigger central and peripheral nervous system mechanisms which is responsible for activation of mediators of innate immunity [108] such as TNF-α, IL-1β and IL-8 [1, 27, 29], promoting inflammatory response and neuro-inflammation.

The nervous system is divided into the CNS, responsible for pain transmission through the ascending and descending neural pathways, and the peripheral nervous system (PNS), responsible for activation of innate immunity inflammatory mediators, such as bradykinin, histamine, serotonin, TNF-α, cytokines and IL. Some authors defend the existence of an apparent dysfunction in ascending and descending neural pathways in FM patients, which would lead to an increased response mediated by amplification of CNS signalling. Simultaneously, PNS would promote the release of inflammatory mediators, which translate systemic and neuro-inflammation [109]. Under inflammatory conditions, harmful stimuli can enhance the pain sensation, i.e. hyperalgesia. In contrast, reducing an inflammatory environment may help alleviate the sensation of pain [110]. Thus, the exclusion of potentially inflammatory foods and the increased intake of foods with anti-inflammatory potential may have attenuated systemic inflammation, allowing a reduction in the pain sensation.

Several bioactive compounds and nutrients can modulate the inflammatory response [111, 112]. It is well known the potential anti-inflammatory effects of Omega 3 fatty acids, vitamin C, phenolic compounds, vitamin D and zinc, while excess iron, *trans* fatty acids, alcohol and a high glycaemic load are associated with increased inflammation [113]. In 2009, Cavicchia and colleagues undertook a systematic review of all studies to date that provided information on the inflammatory potential of foods and active food compounds and built the "Dietary Inflammatory Index**"** [63]. In this index constituted by 45 nutritional compounds, the authors identified *trans* fatty acids and saturated fat, iron and cholesterol as potentially inflammatory. On the other hand, they considered as potentially anti-inflammatory components curcumin, green tea, flavonoids, anthocyanins and oregano. In this context, the anti-inflammatory nutritional approach employed in the present study may have contributed to reduce the systemic inflammatory process present in FM and could provide an explanation of the mechanisms behind our findings. We also suggest that anti-inflammatory dietary intervention could also allow a more attenuated immune response.

Additionally, it is known that the existence of an inflammatory environment promotes the production of ROS and reactive nitrogen species (RNS), which, alongside with an inadequate antioxidant activity, may lead to oxidative stress [114, 115]. Oxidative stress has been recently identified in FM patients, through an increase in plasma levels of lipid peroxides and a decrease in the total antioxidant capacity or a decrease in the plasma concentration of superoxide dismutase and catalase enzymes [114]. In its turn, it is known that increased oxidative stress increases the expression of pro-inflammatory cytokines, such as TNFα and IL-1β, through activation of different kinases involving pathways and transcription factors like Nuclear factor kappa B (NF-κB) [116], amplifying the inflammatory environment already present in these patients, consequently increasing the sensation of pain [117, 118]. In this sense, the promotion of antioxidant intake in the anti-inflammatory diet performed by the intervention group may also have helped to reduce possible oxidative stress, consequently reducing inflammation and pain.

On the other hand, intestinal microbiota has been related to inflammatory diseases by several authors [23, 26]. Intestinal inflammation can alter the composition of the gut microbiota, which further exacerbates inflammation [119]. The disruption of normal mucosal immunity toward the commensal microbiota, in the presence of dysbiosis, leads to continuous microbial antigenic

stimulation and contributes to chronic intestine inflammation [120]. Thus, the first month of low FODMAPs diet, followed by a diet rich in fruits and vegetables, and low in sugar and ultra-processed foods, which, consequently, guarantee a healthy intestinal microbiota, may have possibly treated the dysbiosis and, consequently, contributed to a reduction of possible of low-grade systemic inflammation.

Effect on gastrointestinal symptoms

Gastrointestinal symptoms improved both in intervention and control group. Only two of the clinical trials performed to date have evaluated gastrointestinal disturbances and reported the impact of a dietary intervention in these symptoms [56, 57]. The low FODMAPs diet showed a reduction in gastric pain and intestinal changes in IBS-SSS, with a reduction in 50% of symptoms after a 4 week-intervention [56]. The gluten-free diet showed no significant differences in GI symptoms between intervention and control groups, at the end of the intervention [57].

Gastrointestinal disorders are a common symptom in FM patients. Some authors refer the presence of intestinal dysbiosis and SIBO in these patients [20, 22, 37]. Dysbiosis and metabolic endotoxemia are associated with westernized dietary pattern rich in ultra-processed products, *trans* fatty acids, sugars and refined flour, along with stress and physical inactivity [33, 121]. As consequence, bacteria overgrowth and release of endotoxins, hydrogen sulfide, phenols, ammonia and indoles, expose intestinal mucosa and the host to harmful effects [121, 122]. The FODMAPs mechanism of action is linked to the stimulation of mechanoreceptors as a response to luminal distension from a combination of increased luminal water content from the osmotic effect, especially in the small intestine, and from the release of hydrogen and ammonia from the bacterial fermentation of saccharides. Such stimulation can lead to ascending messages that might be interpreted as abdominal pain or bloating, increased abdominal distension, changes in intestinal motility, and visceral sensitivity due to possible excessive production of short-chain fatty acids [122]. In this context, limiting the intake of the most fermentable carbohydrates may have potentially alleviated FM symptoms, by reducing gases formation.

We suggest that the first month of low FODMAPs diet may have been crucial to reduce SIBO and to optimize intestinal microbiota, allowing a greater efficacy of the posterior anti-inflammatory

approach, and possibly of the pharmacological therapy that patient were already being subjected. The reduction of low-grade inflammation may be the explanation for the symptom's improvement experienced by the intervention group.

Additionally, the whole interventions provides essential nutrients to a healthy intestinal microbiota, enabling the treatment of possible dysbiosis and reducing low-grade systemic inflammation that may exist in patients with FM [26, 43]. The improvement in the composition of the intestinal microbiota allows an optimization of its functions, namely the production of short-chain fatty acids (SCFA). SCFA, specially butyrate, exert anti-inflammatory effects via regulating the intestinal macrophage function as a histone deacetylase (HDAC) inhibitor, suppressing the NF-κB pathway activation, and inducing the expression of IL-10 [120].

The improvement observed in the control group is probably due to the impact of the WHO recommendations for healthy diet being, in itself, an enhancement in the diet of study participants. However, the intervention group obtained a greater improvement in this parameter, with a significant difference still being verified between the two groups at the end of the study.

Effect on fatigue

In respect to fatigue, it was observed a statistically significant improvement in symptoms assessed by the FSS questionnaire in intervention group, at the end of the study. Control group also showed a statistically significant improvement in this parameter. However, the improvement in this group was less pronounced. Moreover, the difference in improvement between the groups was statistically significant.

These results are in agreement with other studies. One of the hypocaloric diet studies showed lower score of fatigue dimension of FIQ after 6 months of intervention, compared to control group at the endpoint [54]. A vegetarian diet also revealed similar results between intervention group and control group [59]. The remaining interventions did not show any significant differences between dietary intervention and fatigue.

The diet adequacy regarding vitamins and minerals of the study participants could be one factor for the fatigue improvement. The restriction in low nutritional density foods, such as ultra-

processed products rich in sugars and poor-quality fats, allowed an optimization of the intestinal microbiota [30], which may have promoted an improved absorption of vitamins, minerals and other bioactive compounds. Additionally, clinical studies demonstrated that some specific foods and nutrients that were included in the anti-inflammatory diet, such as whole grains, polyphenol-rich vegetables, and omega-3 fatty acid-rich foods, have been associated with a possible improve disease-related fatigue symptoms [123].

However, fatigue does not seem to have an exclusively inflammatory component, being accepted as a multi-factorial syndrome. Thus, despite dietary and nutritional anti-inflammatory strategies, a multi-model lifestyle approach seems to be the most suitable [123].

Effect on sleep quality

In this study, it was observed an improvement in sleep quality evaluated through PSQI in both groups, after intervention. Additionally, the results of the between-group analysis of post-intervention – baseline difference was also significant, indicating that intervention group improved more than control group.

These results are in accordance with a 6-month hypocaloric diet study, where intervention group showed significantly lower PSQI score compared to control group [54]. On the other hand, a gluten-free intervention showed no significant effect in sleep quality [57].

Sleep corresponds to a natural and essential metabolic phenomenon, which involves two mechanisms: the endocrine production of the hormone melatonin, resulting from the conversion of serotonin in the absence of light, and the accumulation of adenosine during the day [124]. Sleep is composed of two distinct phases, each lasting an average of 90-110 minutes: non-eye rapid movement (NREM) and rapid eye movement (REM). NREM sleep is composed of phase 1 (light sleep), 2 (which corresponds to about 50% of total sleep) and 3 (deep sleep or slow wave sleep) [125].

There are several factors that can negatively affect the quality of sleep, such as exposure to artificial light, in particular to the blue-light spectrum, the existence of irregular hours and shift work and the presence of a stressful life [126].

On the other hand, dietary pattern also seems to have a strong influence. Caffeine and the use of energy drinks are sleep disruptors, once they link to adenosine receptors promoting alertness [127]. Increasing carbohydrate intake at the last meal of the day appears to increase REM sleep duration and facilitate sleep induction. It happens through branched-chain amino acids (BCAAs) mobilization into muscle, due to insulin increase, allowing the tryptophan, necessary for serotonin synthesis, to cross the blood-brain barrier, enhancing melatonin production [125]. The fact that there was no restriction of these foods in this study may have contributed to the improvement of sleep quality.

Additionally, the increase in pro-inflammatory cytokines such as IL-6 and TNF-α are associated with a reduction in the duration of slow-wave sleep, worsening its quality [126]. These biomarkers are elevated in the presence of obesity, which is one of the reasons why excess weight may compromise sleep quality [128]. However, in our study we found an improvement in sleep quality in the absence of BMI variation. Instead, the possible reduction in inflammation in study participants, which may not had been detected in our study due to the insufficient specificity of CRP and ESR, may be the reason why an improvement in sleep quality occurred. In addition, sleep disturbances can, in its turn, induce the expression of pro-inflammatory cytokines, such as CRP, IL-6 and TNF-α, amplifying inflammation [129], so the improvement in sleep may also have promoted a reduction in inflammation, contributing to the relief of disease symptoms in study participants.

Effect on quality-of-life

Intervention group experienced a significant improvement in quality of life at the end of the study.

Other studies have evaluated the impact of dietary interventions on fatigue. In a raw vegetarian diet study [59], patients revealed an improvement in vitality, mobility, emotional health and mental parameters assessed through SF-36 questionnaire, comparing baseline and endpoint after 7 months. In the same study, it was used another quality of life assessment tool, the Health Assessment Questionnaire (HAQ), in which similar results were found [59]. Additionally, a hypocaloric diet study reported a significant improvement in quality of life, assessed through

Quality of Life (QOL) questionnaire, but no significant differences in HAQ, after 5 months intervention [53].

Quality of life is a parameter that is influenced by sleep, fatigue and pain. By observing the improvement in sleep and fatigue in the intervention group, it would be expected that the quality of life would also have improved in these patients. In fact, the SF-36 questionnaire assesses the mental and physical component [101], and the intervention group significantly improved on both components. The improvement in the quality of life of the participants in the intervention group corresponds to the culmination of the results of the other variables, possibly due to the cumulative effect of the improvement observed in other outcomes.

Inflammatory biomarkers

In respect to inflammatory biomarkers, hs-CRP and ESR did not change between groups nor within groups, in the end of the intervention.

The presence of systemic inflammation in FM has been identified by several authors [2, 7], so, contrary to what have been observed, it would be expected a variation in inflammatory parameters.

Nevertheless, this aspect is still not clear. In one study, after a hypocaloric diet, intervention group showed significantly lower CRP, along with IL-6, compared to healthy control group [54]. However, the sample consisted in obese FM patients. IL-6 is known to be associated with obesity [130], a condition where chronic inflammation is prevalent [131], so weight reduction may have had a positive impact on reducing inflammation, visible in the improvement of biomarkers. On the other hand, the study lasted 6 months, which may also have been relevant in the observation of changes in these biomarkers, compared to our study.

Additionally, a systematic review by Sanada and colleagues concluded that there were no significant changes in CRP after non-pharmacological interventions in FM patients [132]. CRP is a non-specific biomarker, which may be sensitive to other pathologies or conditions of these patients [102]. Other studies evaluated the impact of dietary interventions on other inflammatory cytokines, namely IL-6, IL-8, IL-1β, IL10, TNF-α, Interferon-gamma (INF-γ), and Monocyte chemoattractant protein 1 (MCP1), and observed changes in IL-6 and IL-8. In fact, in

their systematic review, Sanada and colleagues found increased expression of IL-6 and IL-8 [132], also confirmed in a meta-analysis carried out by Uceyler and colleagues [51].

In this sense, it would be important to study the possibility of differences in more specific inflammatory biomarkers after dietary intervention, such as IL-8.

5.2. Overall impact of the proposed dietary approach

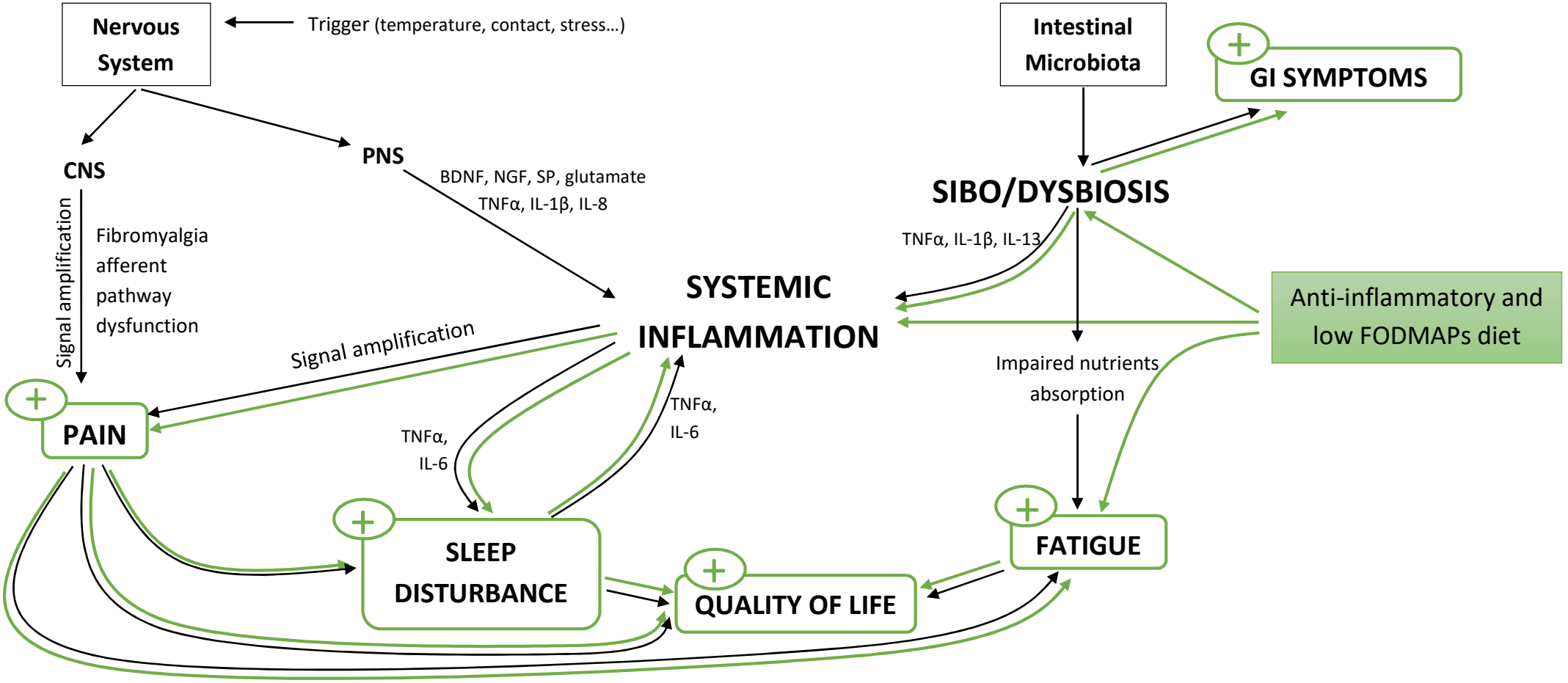
Despite the unknown FM pathophysiology, it has been suggested that genetic predisposition and stressful life events may trigger central and peripheral nervous system mechanisms which is responsible for activation of mediators of innate immunity [108] such as TNF-α, IL-1β and IL-8 [1, 27, 29], promoting inflammatory response and neuro-inflammation. Additionally, the presence of intestinal dysbiosis and SIBO in these patients has been widely discussed [22, 43, 133]. Symptoms of the development of SIBO, namely bloating, abdominal pain and constipation, are very common in these patients [22]. Moreover, changes in the intestinal microbiota compromise the absorption of nutrients and bioactive compounds, essential for the energy function and correct functioning of the organism [134].

Figure 3 represents the possible mechanisms through which the anti-inflammatory and low FODMAPs intervention may have contributed for the improvement of FM symptoms. The activation of the nervous system in the presence of a certain trigger, such as temperature (heat or cold), physical contact or stress, activates two responses: 1) the central nervous system, which translates a pain signal through the afferent pathways; 2) the peripheral nervous system, which induce the production of molecules that promote an inflammatory response [109]. In the presence of an inflammatory environment, the sensation of pain increases [110]. Additionally, a change in the intestinal microbiota composition (i.e. dysbiosis) and/or SIBO, commonly observed in FM patients [20, 22, 27], also increases intestinal inflammation [26, 43]. This condition, to which can be added the possible dysfunction in the afferent pain pathways that seems to exist in FM patients, may amplify the sensation of pain. In the presence of dysbiosis, the absorption of vitamins, minerals and other nutritional compounds may be compromised [30], which can interfere with energy production, reflected in a situation of accentuated fatigue. It is also known

that the presence of pain may be associated with fatigue and may negatively influence the quality of sleep. In its turn, the sleep disorder may also promote the production of inflammation-promoting molecules [126, 129].

A diet that restricts foods with pro-inflammatory potential, and that promotes the intake of foods with anti-inflammatory potential, could potentially have reduced low-grade inflammation, promoting the reduction of pain associated with FM. Moreover, the first month of low FODMAPs diet may have possibly reduced SIBO and optimized intestinal microbiota, allowing a greater efficacy of the posterior anti-inflammatory approach, and possibly of the pharmacological therapy that patients were already being subjected. Additionally, the improved in intestinal microbiota composition and function may have possibly potentiate the better absorption of vitamins, minerals and other food components essential to energy and metabolic function. The sequence of the dietary approaches carried out by the intervention group may have had an important contribution on the symptom's improvement.

Figure 3. Anti-inflammatory and low FODMAPs diet proposed mechanisms of action in fibromyalgia Patient Reported Outcomes



CNS – Central Nervous System; PNS - Peripheral nervous system; GI- Gastrointestinal; SIBO – Small intestinal bacterial overgrowth; BDNF – Brain-derived neurotrophic factor; NGF; SP – Substance P; TNFα – Tumor necrosis factor α; IL-1β - Interleukin-1β; IL-8 - Interleukin-8; IL-13 - Interleukin-13; IL-6 – Interleukin-6.

Every study carried out so far tested the effect of dietary strategies that test an isolated dietary component. In the present study, we used an integrative nutritional and dietary approach, which included anti-inflammatory components and excluded the pro-inflammatory ones, therefore promoting more consistent results. In fact, the absence of individual significant nutritional predictors of the PRO, namely monosaccharides, disaccharides, dietary fiber, omega-3 fatty acids and omega-6 fatty acids, reflects that the interventions with a reductionist nutritional approach, focusing on single nutritional factors may not be enough to improve FM symptoms. Several authors defend that the effect of the overall diet or a dietary pattern appears to have more impact in chronic diseases risk than looking for isolated nutrients [64, 135]. To the best of our knowledge, this is the first study that brings together the multiplicity of food characteristics and nutritional factors with plausibility to improve FM symptoms.

It was possible to observe that, the impact of the intervention on FM symptoms was beneficial in the intervention group regardless of age, disease duration, BMI variation and body fat mass variation between baseline and post-intervention. This fact suggests, contrary to what has been suggested elsewhere [64], that a hypocaloric diet and weight management may not be enough to improve FM symptoms.

There were no significant differences between post-intervention and baseline moments when the impact of the variation in the intake of each nutrient *per se* on FM PRO was tested. Additionally, the effect of the intervention between groups remains significant for pain and functional repercussion and gastrointestinal symptoms, after a multivariate analysis.

Despite the proposed dietary restrictions, the compliance of the participants is confirmed, since exclusion of gluten, sugar, ultra-processed products and dairy in control group was confirmed at the end of the intervention. Therefore, the application of this nutritional strategy in clinical practice seems to be feasible.

5.3. Study limitations

Some limitations were identified during the study. The lack of a blood test for a low-grade inflammation specific cytokine such as IL-8, which has been associated with FM by several authors [51, 132], makes impossible to objectively determine the symptoms improvement mechanisms or to confirm the reduction in low-grade inflammation. Through the evaluation of

CRP and ESR, we aimed to investigate the presence of inflammation in FM. CRP is an acute-phase inflammatory protein, while the ESR increases and decreases very gradually, over days [104]. The combination of the two parameters could theoretically bring very pertinent information about the impact of the diet. However, the absence of results in these parameters may be due to their unspecificity.

Additionally, the absence of assessment at the end of the first month of intervention makes it impossible to objectively assess the impact of low FODMAPs diet alone, as well as the real need to carry it out in this context.

Thirdly, it would be equally important to replicate this study, in order to amplify the sample. The results obtained in our study were statistically significant, and the effect of the intervention between groups remained significant for disease functional repercussion (assessed through FIQR), pain (assessed through VAS), and gastrointestinal symptoms (assessed through VAS_GI) after a multivariate analysis. However, the impact of the study on clinical practice could be even greater if it were possible to amplify the sample.

5.4. Final conclusions

After the anti-inflammatory and low FODMAPs nutritional intervention, there was an improvement in FM symptoms, namely pain, fatigue, gastrointestinal symptoms, quality of sleep and quality of life in intervention group.

The impact of the anti-inflammatory and low FODMAPs diet seems to be diverse. The low FODMAPs diet was essential to solve the gastrointestinal symptoms. Additionally, the optimization of intestinal microbiota may have had a key role in the attenuation of low-grade inflammation, reducing pain and improving functional repercussion. Also, it allows a better absorption of essential nutrients which may impact in fatigue.

Our results provide a novel dietary intervention approach that combines nutritional and dietary strategies with anti-inflammatory potential. In addition, our study considered a wide variety of outcomes, assessed through validated instruments, to broaden the ability to assess typical FM symptoms.

The present study allows us to conclude that an anti-inflammatory and low FODMAPs diet improved PRO in this sample of FM patients, which may represent a relevant complement to the pharmacological therapy.

6. Manuscripts

6.1. Manuscript I

Silva AR, Bernardo MA, Costa J, Cardoso A, Santos P, Mesquita, MF, Vaz Patto J, Moreira, P, Silva, ML, Padrão P

Dietary interventions in fibromyalgia: a systematic review

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REVIEW ARTICLE

Dietary interventions in fibromyalgia: a systematic review

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ABSTRACT

Fibromyalgia (FM) is a chronic non-degenerative disease, whose nutritional therapy seems controversial. This systematic review aimed to synthesize the knowledge about the effect of dietary interventions on patient-reported outcomes (PRO) and inflammation in patients with FM. Six electronic databases – PubMed, BioMed Central, Cochrane library, EMBASE, LILACS and ISI – were searched for clinical trials, in which a dietary intervention in patients with FM diagnosed was conducted. Quality of evidence assessment was measured in accordance with GRADE methodology. Seven clinical trials – 3 randomized controlled trials, 1 unrandomized clinical trial and 3 uncontrolled clinical trials were identified. Dietary approaches included gluten-free diet ($n = 1$), raw vegetarian diet ($n = 2$), low Fermentable oligo-, di- and monossacharides, alcohols and polyols (FODMAPs) diet ($n = 1$), hypocaloric diet ($n = 2$) and monosodium glutamate- and aspartame-free diet interventions ($n = 1$). The major PRO were pain and functional repercussion, with 5 out of 7 studies reporting an improvement. The progress in secondary outcomes was reported for fatigue (2/5 studies), sleep quality (2/3 studies), depression and anxiety (3/6 studies), quality of life (4/5 studies), gastrointestinal symptoms (1/2 studies) and inflammatory biomarkers (1/1 study). However, according to Cochrane Risk of Bias, these studies had poor statistical quality. Well-designed studies should be performed to investigate the dietary interventions effect on FM.

KEY MESSAGES

- Fibromyalgia (FM) is a chronic non-degenerative disease, whose nutritional therapy seems controversial but promising.
- Pain and functional repercussion in FM patients seem to improve with a hypocaloric diet, a raw vegetarian diet or a low FODMAPs diet, as much as quality of life, quality of sleep, anxiety and depression and inflammatory biomarkers.
- Existing studies in this subject are scarce and low quality, which does not allow conclusions to be drawn.

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Dietary interventions; fatigue; fibromyalgia; pain; systematic review

Introduction

Fibromyalgia (FM) is a chronic non-degenerative disease of unknown aetiology that mostly affects women, with a prevalence range between 0.5 and 2% worldwide [1] estimated at 1.7% in Portugal [2]. The diagnosis is based on the criteria of Rome III of the American College of Rheumatology (ACR), reviewed in 2010 [3].

The main symptoms of FM are musculoskeletal pain and chronic fatigue. Patients usually also refer nonrestorative sleep, morning stiffness, depression, anxiety [1] and gastrointestinal (GI) symptoms similar to irritable bowel syndrome (IBS) [4], strongly compromising their quality of life. All these patient's reported outcomes (PRO) are evaluated in clinical practice, through questionnaires which are considered subjective. However, changes in biomarkers, particularly inflammatory cytokines were also described. A meta-analysis with 25 clinical trials and 1255 FM patients revealed a higher plasma interleukin (IL)-6 in these patients, compared to healthy controls [5]. Additionally, several studies showed an association between FM and intestinal inflammation [4,6–8],

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through a slight but significant plasma pro-inflammatory cytokines increase [9], suggesting a low-grade inflammation in these patients, associated with altered intestinal microbiota and dysbiosis [10,11].

Medical therapy consists mainly of analgesic, muscle relaxants and non-steroids anti-inflammatory drugs (NSAID), but it seems not to completely resolve the symptoms of the disease [1,12]. Additionally, the modification of intestinal microbiota composition described in these patients, emerges as an opportunity to intervene through dietary approaches. However, according to the literature, the effect of nutritional interventions on FM remain controversial. Thus, the aim of this systematic review was to synthesize the knowledge about the effect of nutritional interventions on the PRO and inflammation in patients with FM.

Material and methods

This review was performed according to Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [13].

Data sources and study selection

A systematic search was conducted by three independent researchers (AS, AC and PS) in PubMed, BioMed Central, Cochrane library, EMBASE, LILACS and ISI databases, using as keywords the terms "fibromyalgia [all fields] or "fibromyalgia" [MeSH Terms] and "diet" or "diet therapy" or "nutrition" or "sibo" or "small intestinal bacterial overgrowth" or "microflora" or "microbiota" or "intestinal microbiota".

Intervention studies investigating the association between diet and FM that were published from January 1990 to April 2018 were included. The reference lists of included articles were screened manually for additional studies. Disagreements were resolved by consensus.

The last search was conducted on 14 May 2018.

Study design and eligibility criteria

Clinical trials including adult human populations with FM diagnosed according to ACR criteria revised in 2010, within which a dietary intervention was implemented, were considered eligible. No restrictions were imposed on language.

Studies with interventions other than only dietary interventions, such as acupuncture, physical exercise, quiropractice, pharmaceutical interventions, among others, were considered not eligible. Studies including dietary supplementation were excluded. Studies that included patients diagnosed with FM combined with other disorders, such as rheumatoid arthritis, lupus or irritable bowel syndrome, were also excluded.

Data extraction

After selecting the eligible studies, the following information was extracted from each study: name of the first author, year of publication, study design, sample size, characteristics of participants (sex and age), dietary intervention protocol, outcomes and results.

The primary PRO of interest for this study were pain and functional repercussion. The secondary outcomes were fatigue, quality of sleep, quality of life, anxiety and depression and GI symptoms. The presence of inflammation assessed with biomarkers was also an outcome of interest.

Risk of bias and grading system

The risk of bias of the individual studies was assessed through The Cochrane Collaboration's tool for Assessing Risk of Bias in Randomized Studies (ROBIS) [14] and Risk Of Bias In Non-randomized Studies - of Interventions (ROBINS) [15]. Aspects of methodological quality, such as participant selection, classification of interventions and deviations from intended protocol, measurement of outcomes and selection of reported results were evaluated.

Risk of bias was included in the GRADE assessment, in order to assess the quality of evidence, evaluating inconsistency, indirectness of evidence, imprecision and publication bias.

Results

Overview of included studies

After removing duplicates (n = 206), a total of 972 studies were identified. Of these, 954 were excluded due to a non-clinical trial (n = 86), to include patients with other diseases besides FM (n = 797), to include animals (n = 4) or children (n = 1) and to use other interventions besides dietary therapy (n = 66). Eighteen complete articles were included and evaluated, among which 11 were excluded for the following reasons: non-eligible study type (case report, n = 3; only abstract publication, n = 1), duplicated study (n = 3), selected outcomes not included (n = 1), no dietary intervention (n = 1) and patients with other diseases besides FM (n = 2). A total of 7 clinical trials

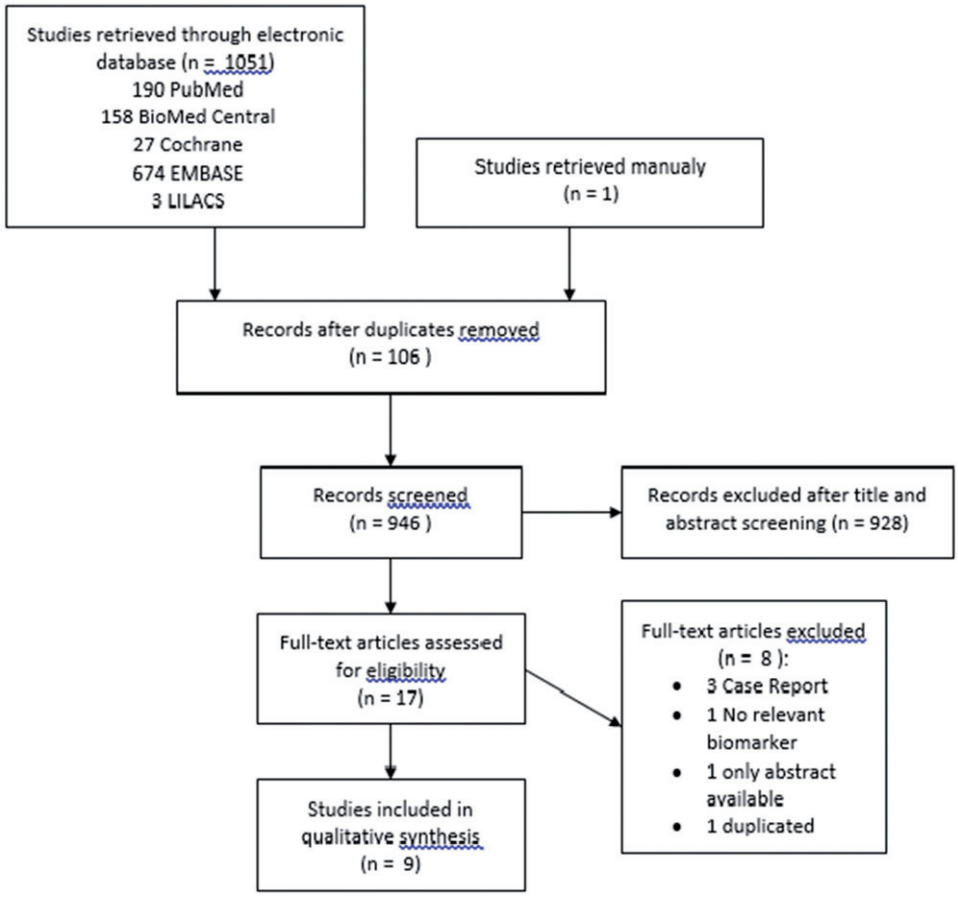

Figure 1. PRISMA flow chart: summary of evidence search and selection.

were included. These results are presented in the Summary of Evidence Search and Selection, which is based on PRISMA flow chart (Figure 1).

From the 7 clinical trials included in this review, 3 were Randomized Controlled Trials (RCT) [16–18], 1 was a Controlled Clinical Trial, Unrandomized (CCT) [19], and 3 were Uncontrolled Clinical Trials (UCT) [20–22].

Included study characteristics

The controlled studies included 266 FM patients (132 in the intervention group and 134 in the control group), of which 255 were women. The UCT studies included 82 FM patients, all women. The mean sample size was 49.7 FM patients, and the time of follow up ranged from 4 weeks to 7 months. The age of the patients ranged from 39.5 to 54.5 years.

The 7 included studies presented distinct dietary interventions: diet low in foods rich in FODMAPs (fermentable oligo-, di- and monossacharides, alcohols and polyols) [20]; gluten-free diet [16]; monosodium glutamate- and aspartame-free diet [17]; hypocaloric diet [18,22]; and raw vegetarian diet [19,21]. The studies used different methods to evaluate the effect of the intervention in PRO and biomarkers parameters. Table 1 summarizes the characteristics of each intervention, in respect to sample size, study design, methodology and results.

The low FODMAPs diet intervention was characterized by an exclusion of all dairy products; all cereals except rice; cashew; all fruit other than banana, citrus, pineapple, red berries, strawberries and kiwi; all vegetables other than pumpkin, cabbage, lettuce, tomato, carrot and cucumber, for a 4 week period [20]. The aim of this study was to examine the effects of a low FODMAPs diet in the PRO, mainly pain, quality of life and GI symptoms.

The hypocaloric diet interventions considered the hypothesis that a weight loss could beneficiate FM symptoms, particularly pain. This intervention was characterized by an ingestion of 1200 kcal/d distributed as 20% protein, 50% carbohydrates and 30% of fats, in the form of vegetables, fruit, whole cereals and light dairy. One study used a group approach methodology [22], and the other used a regular personal dietary plan implementation [18].

Table 1. Characteristics and results of included studies.

Reference	Study design and participants	Intervention	Outcome measures	Results
Low FODMAPs diet				
[20]	UCT Female FM patients ($n = 38$) Age: 51 ± 10 years	Diet low in FODMAPs (LFD) 4 weeks (31 women completed the intervention)	– Questionnaires: FSQ, FIQR, IBS-SSS, EQ-5D, VAS (abdominal pain and somatic pain); – evaluation of satisfaction with diet;	Comparison before and after the intervention: – ↓ pain associated with FM, fatigue, gastric pain and intestinal changes after 4 weeks ($p < .01$) – ↓GI symptoms ($p < .01$) – ↑ mobility and ↓ discomfort in T2 ($p < .05$) – no significant differences in quality of life
Gluten-free diet				
[16]	RCT Diet Group $n = 35$ female FM patients Age: 52 years Control Group $n = 40$ female FM patients Age: 53 years	Gluten-free diet (GFD) VS Hypocaloric Diet (HD); 6 months	– Anthropometric data: weight, BMI, waist perimeter – Biochemical analyzes – List of NCGS symptoms (GI symptoms, extraintestinal and FM-like) – Questionnaires: FIQR, BPI, PSQI, BDI, STAI, SF-12, PGI-I	Comparison before and after the intervention: – no significant differences in pain associated with FM and GI, extraintestinal and FM-like symptoms in GFD and HD Comparison between groups: – no significant differences
Hypocaloric diet				
[18]	RCT Diet Group $n = 43$ FM obese patients (37 female Age: 44.8 ± 13.6 years Control Group $n = 43$ FM obese patients (38F) Age: 46.3 ± 14.4 years	Hypocaloric diet (1200 kcal/d: 20% Prot; 50% CH; 30% Fat) (G1) VS isocaloric diet (G2) 6 months	– Anthropometric data: weight, BMI, waist perimeter – Questionnaires: FIQ, TP, BDI, PSQI – Biomarkers: IL6 and PCR	Comparison before and after the intervention: – ↓ pain associated with FM ($p < .05$), localised pain ($p < .001$), fatigue ($p < .05$) and depression ($p < .001$) in G1 – ↓ IL6 and PCR ($p < .05$) in G1 Comparison between groups: – ↓ IL6 ($p = .034$) and PCR ($p = .07$) in G1 vs G2
[22]	UCT Female FM patients ($n = 48$) Age: 54.5 ± 8.1 years	Hypocaloric diet (weight loss programme with weekly group sessions) 5 months (31 women completed the intervention)	– Anthropometric data: weight, BMI, waist circumference – Questionnaires: FIQ, HAQ, MPI, BDI, STAI, QOL, BSQ – Food diary (5 days)	Comparison before and after the intervention: – ↓ pain associated with FM ($p = .00$), severity ($p = .04$) and day-to-day pain interference ($p < .001$) – ↑ body image ($p < .001$) and quality of life ($p < .001$) – ↓ anxiety ($p < .001$) and depression ($p < .001$) Correlation between variables: – Positive correlation between ↓ BMI and ↓ FM-associated pain ($p = .02$) and day-to-day pain interference ($p < .001$)
Vegetarian diet				
[19]	CCT Diet Group $n = 18$ FM female patients Age: 51 years Control Group $n = 15$ FM female patients Age: 52 years	Vegan Diet (raw veg, fruit, whole grains, oilseeds and legumes) (VD) VS Omnivorous Diet (OD) 3 months	– Questionnaires: TP, VAS pain, BDI, HAQ – Biomarkers: Haematocrit, ERS, total cholesterol, urinary sodium – Food diary (5 days)	Comparison before and after the intervention: – ↓ pain ($p < .005$) but not significant of the PT ($p = .07$) – ↑ autonomy ($p = .03$), sleep quality ($p = .01$), morning stiffness ($p = .0001$) – ↓ total cholesterol ($p < .003$) and urinary Na ($p = .0001$)

(*continued*)

Table 1. Continued.

Reference	Study design and participants	Intervention	Outcome measures	Results
				– no significant statistical differences on depression, ERS and haematocrit
[21]	UCT FM patients ($n = 30$), (28 female)	Raw vegan diet (raw veg, fruit, whole grains, oilseeds) 7 months (20 adults completed the intervention)	– Questionnaires: FIQR, SF36, QOL, FFQ	Comparison before and after the intervention: – ↓ pain associated with FM ($p < .05$) – ↑ vitality, mobility, emotional health and general well-being after 7 months ($p < .01$) – ↑ general quality of life ($p < .05$)
Monossodium Glutamate and Aspartame-free diet				
[17]	RCT Diet Group $n = 36$ FM female patients Age: 42.3 ± 8.4 years Control Group $n = 36$ FM female patients Age: 39.6 ± 8.2 years	Diet free of monosodium glutamate (G1) VS diet without dietary restrictions (G2, on waiting list) 3 months	– Questionnaire: VAS pain – Food diary (3 months)	Comparison between groups, before and after the intervention: – no significant statistical differences

BDI: Beck Depression Inventory; BMI: Body Mass Index; BPI: Brief Pain Inventory; BSQ: Body Shape Questionnaire; CCT: Controlled Clinical Trial; EQ-5D: EuroQol-5Dimension; FFQ: Food Frequency Questionnaire; FIQ: Fibromyalgia Impact Questionnaire; FIQR: Fibromyalgia Impact Questionnaire Revised; FSQ: Fibromyalgia Survey Questionnaire; HAQ: Health Assessment Questionnaire; IBS-SSS: Irritable Bowel Syndrome-Symptom Severity Survey; MPI: Multidimensional Pain Inventory; NCGS: Non Coeliac Gluten Sensitivity; PGI-S: Patient Global Impression Scale Severity; PGI-I: Patient Global Impression Scale Improvement; PSQI: Pittsburgh Sleep Quality Index; QOL: Quality Of Fife Survey; RCT: Randomized Controlled Trial; SF-36: Short-Form Healthy Survey; STAI: State Trait Anxiety Inventory; TP: Tender Points; UCT: Uncontrolled Clinical Trial; VAS: Visual Analogic Scale.

In the gluten-free diet intervention [16], patients were randomly distributed in two groups: intervention group engaged in a gluten-free diet for 6 months, avoiding wheat, rye, barley and oat; control group underwent a hypocaloric diet, described previously.

In the vegetarian diet interventions, patients were instructed to embrace a raw, low-salt ingestion of vegetables, legumes, fruit, whole cereals and nuts. In one study patients were distributed, according to their own preference, in two groups: intervention group and control group, which continued to have an omnivorous diet [19]. The other study had no control group [21].

In the monosodium glutamate- and aspartame-free diet [17], patients were randomly distributed in two groups: intervention group employed monosodium glutamate- and aspartame-free diet for 3 months, and the control group was placed on a waiting list.

Overview of the outcomes (PRO and biomarkers)

Five of the 7 clinical trials evaluated the pain and functional repercussion as primary efficacy variable, through Fibromyalgia Impact Questionnaire (FIQ) [18,21,22] or Revised Fibromyalgia Impact Questionnaire (FIQR) [16,20]. To evaluate the intensity of pain, 2 studies applied Visual Analogic Scale (VAS) [17,19], other Multidimensional Pain Inventory (MPI) [22], and other the Brief Pain Inventory (BPI) [16]. Examination of Tender Points (TP), was also assessed by 2 studies [18,19].

Secondary outcomes varied according to each study. Fatigue was not evaluated through a specific tool by any study. However, it was considered in 5 studies, as it is referred in one question of FIQ [16,18,20–22]. Quality of sleep was assessed through Pittsburg Sleep Quality Inventory (PSQI) by 2 studies [16,18]. Four studies assessed depression through Beck Depression Inventory (BDI) [16,18,19,22] and 2 assessed anxiety through State-Trait Anxiety Inventory (STAI-I) [16,22]; 1 study assessed both variables through the Five Dimensions Euro – Quality of Life (EQ-5D) [20]. The quality of life was assessed in 5 studies, through EQ-5D [20], Short-Form (SF)-12 [16] or SF-36 [21] and Health Assessment Questionnaire [19,22]. Two studies evaluated GI symptoms through Irritable Bowel Syndrome – Symptom Severity Scale (IBS-SSS) [20] and through a classification of a list of common symptoms based on current literature of Non-Coeliac Gluten Sensitivity (NCGS) [16]. Additionally, 1 study assessed inflammatory biomarkers parameters, namely Interleukin (IL)-6 and C reactive protein (CRP) [18].

Four of the 7 studies applied measures to control the diet compliance, such as food record [17,19,22] or food frequency questionnaire (FFQ) [21].

Effect on pain and functional repercussion

Despite the differences between the dietary approaches, the results in every single study are similar regarding the impact of intervention on these PRO, except for 2 studies, the aspartame- and monosodium glutamate-free diet [17] and the gluten-free interventions [16], which revealed no significant differences between the intervention and the control group.

Intervention with a low FODMAPs diet [20] reduced significantly pain associated with FM: there was a reduction both in FIQR (61.6 vs. 48.1, $p < .01$) and FSQ scores (21.8 vs. 16.9, $p < .01$), between the beginning and the end of the intervention. Additionally, this study showed a significant and positive correlation ($r = 0.36$, $p < .05$) between FIQR and IBS-SSS.

Similarly, hypocaloric diet interventions also showed a significant reduction in pain. In 1 study [22], FIQ scores decreased from 56.7 ± 14.9 to 46.2 ± 18.3 ($p < .001$), after 5 months of the intervention. Also, MPI decreased from 3.8 ± 1.1 to 3.3 ± 1.4 ($p = .04$). In addition, this study showed a direct significant correlation between weight and both MPI ($r = 0.31$, $p < .05$) and HAQ ($r = 0.35$, $p < .05$) [22] at baseline. In the other study [18], after 6 months of hypocaloric diet, the intervention group presented significantly improved FIQ scores compared to the control group (51.6 ± 9.4 vs. 47.0 ± 5.1, $p = .007$).

The vegetarian interventions also revealed an improvement in pain. One vegetarian diet intervention [21] showed a significant reduction in FIQR, after 7 months of intervention (51.4 ± 14.2 vs. 27.6 ± 19.0, $p < .001$). In the other vegetarian study, the authors describe a significant reduction in VAS ($p < .005$), however the score values obtained were not published [19].

Effect on fatigue

As mentioned, fatigue was considered in 5 studies, as it is referred in one question of FIQ [16,18,20–22]. One of the hypocaloric diet studies showed a lower score of fatigue dimension of FIQ after 6 months of intervention, compared to control group (4.7 ± 1.8 vs. 5.8 ± 1.9, $p = .008$) at the endpoint [18]. A vegetarian diet also revealed similar results after 7 months of intervention (7.8 ± 3.2 vs. 4.4 ± 2.8, $p < .05$) [21]. The remaining interventions did not show any significant differences between diet and fatigue.

Effect on sleep quality

After 6 months of intervention with a hypocaloric diet, intervention group showed significantly lower PSQI score compared to control group (4.0 ± 1.9 vs. 5.3 ± 2.4, $p = .006$) [18]. In parallel, a gluten-free intervention showed no significant effect in sleep quality [16].

Another intervention, including a vegetarian raw diet for 3 months, which assessed the quality of sleep through a non-identified questionnaire, reported significant differences after intervention ($p < .001$) [19]. However, the study protocol did not reveal the tool's details, so this result was not considered.

Effect on depression and anxiety

Three studies reported an improvement in depression and anxiety. One hypocaloric approach showed a decrease in BDI scores (17.8 ± 11.2 vs. 9.7 ± 8.4, $p < .001$) and in STAI scores (42.8 ± 11.7 vs. 35.8 ± 11.3, $p < .001$) after intervention, in comparison with the initial scores [22]. Similarly, the other hypocaloric trial revealed a significant difference between intervention and control groups after a 6 months intervention (12.8 ± 5.8 vs. 17.6 ± 7.7, $p = .002$) in BDI [18]. Regarding vegetarian studies, 1 revealed a significant decrease after 7 months intervention, in the depression (5.0 ± 3.0 vs. 2.4 ± 2.5, $p < .05$) and anxiety (5.7 ± 2.7 vs. 3.0 ± 2.3, $p < .05$) dimensions of FIQ [21]. The gluten-free diet [16], low FODMAPs diet [20] and the other vegetarian diet [19] interventions did not show a significant effect on anxiety and depression.

Effect on quality of life

In low FODMAPs diet study [20], EQ-5D score differences before and after intervention had no significant differences. However, the dimensions Mobility and Pain significantly improved (2.7 vs. 2.3, $p = .02$ and 3.5 vs 2.8, $p < .01$, respectively). In a raw vegetarian diet study [21], patients revealed parameters improvement comparing the begin and endpoint, regarding vitality (18.0 ± 14.4 vs. 48.0 ± 28.9, $p < .001$), mobility (36.3 ± 24.3 vs. 60.3 ± 26.7, $p < .001$), emotional health (25.0 ± 26.5 vs. 75.0 ± 25.7, $p < .001$) and mental health (57.2 ± 23.1 vs. 77.0 ± 15.3, $p < .001$) in SF-36 questionnaire. Additionally, there was an improvement in HAQ scores, from 3.9 to 4.7 ($p < .05$) after 7 months, in the same study [21]. On another

vegetarian study, although the score values obtained were not published, the authors reported that the intervention group had better autonomy ($p = .03$) and morning stiffness ($p < .001$) [19] compared with control group, assessed through HAQ. Hypocaloric diet showed a significant improvement in quality of life, assessed through QOL (33.2 ± 26.7 vs. 44.6 ± 29.8, $p = .01$), but no significant differences in HAQ, after 5 months intervention [22]. Gluten-free diet intervention showed no significant differences in SF-12 questionnaire [16].

Effect on gastrointestinal symptoms

Two of the 7 studies evaluated GI disturbances among FM patients, and reported the impact of a dietary intervention in these symptoms [16,20]. A low FODMAPs diet showed a reduction in gastric pain and intestinal changes in IBS-SSS (275.3 vs. 158.1, $p < .01$), with a reduction in 50% of symptoms after a 4 week-intervention [20]. The gluten-free diet showed no significant differences in GI symptoms between intervention and control groups, at the end of the intervention [16].

Inflammatory biomarkers

Only 1 study measured inflammatory biomarkers parameters, namely IL6 and CRP. After a hypocaloric diet, intervention group showed significantly lower inflammation biomarkers compared to control group, namely IL6 (4.1 ± 1.5 pg/ml vs. 3.4 ± 1.4 pg/ml, $p = .03$) and CRP (2.6 ± 1.1 mg/dL vs. 2.0 ± 1.1 mg/dL, $p < .001$) at the end of the intervention [18].

Risk of bias and GRADE

The results after applying the Cochrane Risk of Bias Tool are presented in Table 2. Although the quality of evidence of the studies varied, the Risk of Bias analyzed allowed us to verify a poor statistical quality in most of them. The majority of the studies have a high risk of bias, which decrease the quality of evidence. The risk of bias was integrated into GRADE profile.

GRADE methodology allowed an analysis of the included studies, according to the risk of bias, inconsistency, indirectness of evidence, imprecision and publication bias. According to the nature of a dietary study, it is not possible to blind population. As so, this was not considered a factor to downgrade studies. However, there were some reasons that downgraded the included studies, such as: the small sample size and optimal information size (OIS) never estimated [16–22]; some studies had no control group [20–22] or no randomization [19] or did not use an independent control group (two different interventions are applied) [16]; some studies did not apply intention-to-treat analysis, despite presenting >5% of loss of follow up [19,20,22]; some studies presented heterogeneity of the population in pain level [19] or medical therapy [16], at baseline. This analysis resulted in an evaluation of low to very low uncertainty of evidence, except for one study [18], considered of moderate uncertainty. Table 3 shows a summary of GRADE profile for studies representing each outcome. Risk of bias classification justification is shown in Table 4.

Given the diversity of studies, it was not possible to conduct a meta-analysis.

Discussion

This study reviewed the evidence of dietary interventions effect in PRO and inflammatory biomarkers of FM patients and identified 7 studies that fulfilled the inclusion criteria. To the best of our knowledge this is the first systematic review on dietary interventions effect in this population. According to the results of this review, a hypocaloric diet, a raw vegetarian diet or a low FODMAPs diet may improve pain and functional repercussion in FM patients. However, the fact that the improvement was achieved with different dietary approaches, may lead to the hypothesis that the psychosomatic component of the disease must be taken into account. On the other hand, FM symptoms appear to be associated with several metabolic alterations, namely with regard to changes in the composition of the intestinal microbiota and consequent

Table 2. Cochrane risk of bias of included studies.

	Shapiro [22]	Slim [16]	Marum [20]	Vellisca [17]	Kaartinen [19]	Senna [18]	Donaldson [21]
Randomization sequency generation	X	+	X	?	X	+	X
Allocation concealment	X	?	X	?	X	+	X
Blinding of participants and personal	X	X	X	X	X	X	X
Blinding of outcomes assessment	X	X	X	X	X	X	X
Incomplete outcome data	X	+	X	+	+	+	+
Selective reporting	+	+	+	+	+	+	+
Other bias	X	?	?	?	?	?	?

+: Low risk of bias; X: High risk of bias; ?: Unclear risk of bias.

Table 3. GRADE Profile for studies evaluating the impact of dietary interventions on PRO and inflammation in FM patients.

Dietary interventions compared to usual treatment in Fibromyalgia patients

Certainty assessment							Summary of findings				
							Study event rates (%)			Anticipated absolute effects	
Participants (studies) Follow-up	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall certainty of evidence	With usual treatment	With Dietary interventions	Relative effect (95% CI)	Risk with usual treatment	Risk difference with Dietary interventions
Pain in FM (CRITICAL OUTCOME, assessed with Questionnaires: FIQ, FIQR; Scale from 0–100)											
RCTs 161 (2 studies) (6 months) [18,32]	serious[a,b]	serious[c]	not serious	serious[d]	none	⊕◯◯◯ VERY LOW	83	78	–	–	The mean FIQ in intervention group was **4.27 lower** (7.3 lower to 1.24 lower)
Observational 82 (3 studies) (5–7 months) [21,22]	serious[a,e,f,g,h,i]	not serious	not serious	serious[d]	none	⊕◯◯◯ VERY LOW	–	82	–	–	–
Pain in FM (CRITICAL OUTCOME, assessed with VAS; Scale from 1–10)											
RTCs 105 (2 studies) (3 months) [17,19]	serious[a,e,i,j,k]	serious[l,m]	serious[n]	serious[d]	none	⊕◯◯◯ VERY LOW	51	54	–	–	The mean VAS in intervention group was **0.16 lower** (0.58 lower to 0.26 higher)
Fatigue in FM (CRITICAL OUTCOME, assessed with FIQ and FIQR; Scale from 0–100)											
RCTs 161 (2 studies) (6 months) [18,32]	serious[a,b]	serious[c]	not serious	serious[d]	none	⊕◯◯◯ VERY LOW	83	78	–	–	not pooled
Observational 82 (3 studies) (1–7 months) [20,21,22]	very serious[a,e,f,g,h,i]	not serious	not serious	serious[d]	none	⊕◯◯◯ VERY LOW	–	82	–	–	–
Quality of Sleep in FM (IMPORTANT OUTCOME, assessed with PSQI; Scale from 0–21)											
RCTs 161 (2 studies) (6 months) [18,32]	serious[a,b]	serious[c]	not serious	serious[d]	none	⊕◯◯◯ VERY LOW	83	78	–	–	The mean PSQI in intervention group was **1.22 lower** (2.05 lower to 0.4 lower)
Anxiety in FM (IMPORTANT OUTCOME, assessed with STAI, EQ-5D)											
Observational 62 (2 studies) (1–5 months) [20,22]	very serious[a,e,f,i]	not serious	not serious	serious[d]	none	⊕◯◯◯ VERY LOW	–	62	–	–	–

(*continued*)

Table 3. Continued.

Dietary interventions compared to usual treatment in Fibromyalgia patients

Certainty assessment							Summary of findings				
							Study event rates (%)			Anticipated absolute effects	
Participants (studies) Follow-up	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall certainty of evidence	With usual treatment	With Dietary interventions	Relative effect (95% CI)	Risk with usual treatment	Risk difference with Dietary interventions
Depression in FM (IMPORTANT OUTCOME, assessed with BDI; Scale from 0–50)											
RCTs 194 (3 studies) (3–6 months) [16,18,19]	very serious[a,b,e,j]	serious[c,l,m]	not serious	serious[d]	none	⊕◯◯◯ VERY LOW	98	96	–	–	The mean BDI in intervention group was **0.86 lower** (1.79 lower to 0.07 higher)
Observational 31 (1 study) (5 months) [22]	serious[a,e,f,i]	not serious	not serious	serious[d]	none[o]	⊕◯◯◯ VERY LOW	–	31	–	–	–
Quality of Life in FM (CRITICAL OUTCOME, assessed with SF-12, HAQ)											
RCTs 108 (2 studies) (3–6 months) [16,19]	serious[a,b,e]	serious[c,l,m]	not serious	serious[d]	none	⊕◯◯◯ VERY LOW	55	53	–	–	not pooled
Quality of Life in FM (CRITICAL OUTCOME, assessed with EQ-5D, SF-36, HAQ)											
Observational 82 (3 studies) (4 weeks to 7 months) [20,21,22]	very serious[a,e,f,g,h]	not serious	not serious	serious[d]	none	⊕◯◯◯ VERY LOW	–	82	–	–	–
Gastrointestinal symptoms in FM (IMPORTANT OUTCOME, assessed with IBS-SSS)											
Observational 31 (1 study) (1 month) [20]	serious[a,e,f]	not serious	not serious	serious[d]	none[o]	⊕◯◯◯ VERY LOW	–	31	–	–	-
Inflammation in FM (IMPORTANT OUTCOME, assessed with IL6 biomarkers)											
RCTs 86 (1 study) (6 months) [18]	not serious[a]	not serious	not serious	serious[d]	none	⊕⊕⊕◯ MODERATE	43	43	–	–	The mean IL6 in intervention group was **0.7 lower** (1.31 lower to 0.09 lower)

(*continued*)

Table 3. Continued.

Dietary interventions compared to usual treatment in Fibromyalgia patients											
Certainty assessment							Summary of findings				
							Study event rates (%)			Anticipated absolute effects	
Participants (studies) Follow-up	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall certainty of evidence	With usual treatment	With Dietary interventions	Relative effect (95% CI)	Risk with usual treatment	Risk difference with Dietary interventions
Inflammation in FM (IMPORTANT OUTCOME, assessed with PCR biomarker)											
RCTs 86 (1 study) (6 months) [18]	not serious[a]	not serious	not serious	serious[d]	none	⨁⨁⨁◯ MODERATE	43	43	–	–	The mean PCR in intervention group was **0.6 lower** (1.06 lower to 0.14 lower)

RCTs: Randomized Controlled Trials; CI: Confidence interval.
[a]According to the nature of a dietary study, there isn't possible to blind population. As so, this was not considered a factor to downgrade studies.
[b]Study does not use an independent control group (two different interventions are applied).
[c]Heterogeneity of the population in pharmaceutical therapy, at baseline.
[d]Sample size is always reduced in all studies; the Optimal Information Size (OIS) is never estimated.
[e]Study did not apply intention-to-treat analysis, despite presenting >5% of loss of follow up and/or do not justify missing data.
[f]Study has no control group.
[g]Do not control possible confounding factors, as the increased Omega 3 ingestion, which is known to have an anti-inflammatory potential.
[h]Do not justify missing data.
[i]Do not control possible confounding factors, as medical therapy.
[j]Study has no randomization.
[k]Study does not indicate how the randomization was done.
[l]Heterogeneity of the population in BMI, at baseline.
[m]Heterogeneity of the population in pain level, at baseline.
[n]Study evaluates only pain, ignoring other important symptoms for the patient.
[o]Single study.

Table 4. Summary of author's justification of risk of bias classification.

Bias	Author's judgement	Support for judgement
Randomization sequence generation	High risk	Uncontrolled or unrandomized clinical trial
Allocation concealment	High risk	Open label
	Unclear risk	Authors don't define in the study
	Low risk	Quote: "outcome assessor was unaware of allocation of patients"
Blinding of participants and personal	High risk	Open label
Blinding of outcomes assessment	High risk	Open label
Incomplete outcome data	High risk	Missing data >10% without intention-to-treat analysis
	Low risk	No missing data or, in the presence of missing data >10%, authors describe an intention-to-treat analysis
Selective reporting	Low risk	All pro-specified outcomes were reported
Other bias	High risk	Uncontrol of possible confounders, like medication or diet compliance
	Unclear risk	Authors don't say if possible confounding domain exists

existence of Small Intestinal Bacterial Overgrowth (SIBO) [4,10,11], changes in the hypothalamic axis and increase of cortisol [23,24], mitochondrial dysfunction and oxidative stress [24–27] and alterations in the Central Nervous System, with activation of glial cells in cerebrospinal fluid [28]. In this perspective, a combination of several dietary approaches that could interfere in each metabolic alteration could be a better way to improve the disease symptomatology.

Patients with FM often report specific food intolerances and undertake dietary approaches, seeking an improvement of their symptoms and a better quality of life [29]. In this review, most of the included studies used tools that assess not only the pain associated with FM, but also other common PRO, namely fatigue, quality of sleep, anxiety and depression, general quality of life, and GI symptoms, whereas only one assessed inflammatory biomarkers. This reveals an attempt to better understand the disease and its symptoms, and to meet the needs of these patients, since medical treatment does not appear to be fully effective in eliminating symptoms. The included clinical trials showed a significant improvement in the quality of life [21,22], quality of sleep [18,19], and anxiety and depression [18,21,22]. Additionally, a hypocaloric study showed a reduction in IL6 and CRP after 6 months [18], which reveals an objective positive impact of a weight reduction in decreasing inflammation.

In parallel, high body mass index has been directly and significantly correlated to pain and functional repercussion in FM patients [22], suggesting that obesity could influence the symptoms of the disease. Other authors have postulated that fact previously [30], since adipocytes produce pro-inflammatory cytokines that could prorogate the pain. Furthermore, some studies pointed the existence of an association between FM and intestinal inflammation [1,6,7,31], which suggests that in addition to weight reduction, a diet with an anti-inflammatory potential could contribute to improve disease symptoms.

Moreover, the decrease in GI symptoms associated with a low FODMAPs diet intervention was related with a decrease in pain and functional repercussion [20], revealing a possible association of these symptoms and intestinal microbiota changes.

It is already known that GI symptoms, such as nausea, vomiting and dyspepsia, are very common in patients with FM [1,32,33]. Various authors suggested that the persistence of the described symptoms, along with sleep quality changes, depression and pain, may be related to modifications of intestinal microbiota [1], and consequent existence of SIBO [34–36].

However, it is worth mentioning that the included studies have relevant bias that may limit the interpretation of the results. Given the nature of dietary intervention, it is always impossible to perform a double-blind intervention, which increases the risk of bias. In addition, some studies have other parameters that decrease the quality of the design, namely regarding the lack of control group (n = 3) [20–22] and non-randomization of the sample (n = 1) [19], which allows less control over possible confounding variables.

Furthermore, not only the small size of the total sample, but also the divergences in the methodology used among studies, contribute to the difficulty of obtaining conclusive results. In addition, the fact that the follow-up time for each intervention is diverse, increases the probability of obtaining different effects on the measured outcomes further contributing to inconsistent results, which may hamper a conclusion based on a summary measure of the various studies. Additionally, although the same variables were evaluated in different studies, diverse methods were used to evaluate each of them. This factor may influence results and, consequently, the conclusions, as some methods may enable a more specific or a more comprehensive assessment of a given parameter.

The majority of studies did not take into account possible confounding variables, such as sex, pain level

at baseline and medication, which may potentially confound the association between diet and disease-related variables. Also, in three studies [16,18,20], no methods of controlling diet compliance were applied, which means that is not possible to exclude the hypothesis that the diet has not been fully attained.

In general, the risk of bias allowed to assume a poor statistical quality in most of these studies. Since that, the positive associations between the different dietary interventions and the outcomes should be regarded as potential associations that deserve to be further studied.

Although, dietary interventions seem to be promising as complementary therapies in FM, the results of this review should be interpreted with caution. Well-designed studies are lacking to conclude about the effect of the nutritional interventions on the progression and symptoms of FM.

Conclusion

Pain and functional repercussion in FM patients seem to improve with a hypocaloric diet, a raw vegetarian diet or a low FODMAPs diet. Other PRO, such as quality of life, quality of sleep, anxiety and depression and inflammatory biomarkers also showed a significant improvement with these interventions. However, due to the low quality of the included studies, these promising results should be interpreted with caution, and no quantitative and objective conclusions should be drawn.

The development of well-designed clinical trials in FM patients are needed to conclude about the effect of the dietary interventions on FM patients. Dietary interventions based on scientific evidence, combined with medical therapy could be a strategic approach, in the treatment of FM.

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6.2. Manuscript II

Silva AR, Bernardo MA, Mesquita, MF, Vaz Patto J, Moreira, P, Silva, ML, Padrão, P

A study protocol for a randomized controlled trial of an anti-inflammatory nutritional intervention in patients with fibromyalgia

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Trials

STUDY PROTOCOL Open Access

A study protocol for a randomized controlled trial of an anti-inflammatory nutritional intervention in patients with fibromyalgia

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Abstract

Background: This study aims to analyze the effects of a potentially anti-inflammatory nutritional intervention in disease assessment parameters, inflammatory markers, and quality of life of fibromyalgia (FM) patients.

Methods: A sample of 100 female patients diagnosed with FM, followed up at Portuguese Institute of Rheumatology (IPR) in Lisbon, is being randomly allocated in two groups. Patients in the intervention group are adopting an anti-inflammatory diet, characterized by the exemption of the intake of foods containing gluten, dairy, sugar, and ultra-processed foods, during 3 months. During the first month, a low fermentable oligo-, di-, and monosaccharides and polyols (FODMAPs) diet is implemented, along with the anti-inflammatory diet, followed by the reintroduction of all fruits and vegetables over a consecutive period of 2 months. Patients in the control group are adopting a diet based on general recommendations for healthy eating. The outcomes are pain, fatigue, quality of sleep, quality of life, gastrointestinal symptoms, and inflammation. Before and after the 3 months intervention, and also 1 month after beginning the intervention, the following questionnaires are applied: Revised Fibromyalgia Impact Questionnaire, visual analog pain scale, Brief Pain Inventory,visual analog scale from a list of common gastrointestinal and extraintestinal symptoms in FM, Short Form 36, Fatigue Severity Survey, and Pittsburg Sleep Quality Index. Ultra-sensitive serum C-reactive protein, eritrocyte sedimentation rate, and interleukin-8 are determined. Age, physical activity, anthropometric parameters, and body composition are being collected. Student's *t* test will assess the association between the disease evaluation parameters, the inflammatory markers, and the dietary interventions.

Discussion: The results of this study are expected to determine whether a change in patient nutrition helps to alleviate symptoms, which would optimize medical intervention.

Trial registration: www.ClinicalTrials.gov NCT04007705. Registered on July 5, 2019.

Keywords: Fibromyalgia, Diet, Anti-inflammatory, FODMAPs, Pain, Quality of life, Randomized controlled trial

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Background

Fibromyalgia (FM) is a chronic non-degenerative disease of unknown etiology, with a prevalence range between 0.5 and 2% worldwide [1], 2.1% (95% CI 2.0–2.2) in men and 3.6% (CI 95% 3.5–3.7) in women [2]. In Portugal, the estimated prevalence is 1.7% [3]. The main symptoms of the disease are musculoskeletal pain and chronic fatigue, in addition to nonrestorative sleep, morning stiffness, depression, anxiety [1], and gastrointestinal (GI) symptoms similar to irritable bowel syndrome (IBS) [4]. Medical therapy consists mainly in analgesic, muscle relaxants, and non-steroids anti-inflammatory drugs (NSAID), but it seems not to completely resolve the symptoms of the disease [1, 5].

Recently, several authors showed an association between FM and dysbiosis [6, 7], and in particular with small intestinal bacterial overgrowth (SIBO) [8, 9], characterized by the inappropriate colonization of the distal small bowel with colonic bacteria [10]. A clinical trial with 38 FM women showed that a low ingestion in fermentable oligo, di-, and monosaccharides and polyols (FODMAPs) could improve SIBO, decreasing pain associated with FM, fatigue, gastric pain, and intestinal changes after 4 weeks [11].

Furthermore, other studies revealed a presence of intestinal inflammation [4, 12–14], through a plasma pro-inflammatory cytokines increase [15–17], particularly interleukin (IL)-6 and IL-8 [16, 17], suggesting a low grade inflammation in these patients, associated with dysbiosis [6, 7]. Literature suggests that foods with inflammatory potential, as the ones described in "Dietary Inflammatory Index" [18, 19] could have a critical role in FM symptoms. Additionally, it is also known the pro-inflammatory effect of gluten [20], dairy [21], and ultra-processed foods [22], and on the other hand, the anti-inflammatory potential of omega 3 [23] and antioxidants [24].

In fact, in a systematic review conducted by our team, it was reported that pain and functional repercussion in FM along with quality of life [25–27], quality of sleep [26], anxiety [27], depression [27, 28], and inflammatory biomarkers [28] seem to improve with a hypocaloric diet [27, 28], a raw vegetarian diet [25, 26] or a low FODMAPs diet [11]. However, the existing clinical trials on this subject are scarce and low quality, which does not allow conclusions to be drawn [29]. Additionally, to our knowledge, a nutritional approach involving a combination of several anti-inflammatory dietary factors has never been designed.

Taking those findings together, it seems relevant to test the hypothesis that a dietary intervention which includes potentially anti-inflammatory foods and excludes the potentially pro-inflammatory ones, and that simultaneously allows an optimization of the intestinal microbiota, could reduce intestinal inflammation and dysbiosis, and consequently improve the FM patient's reported outcomes (PRO).

Methods and analysis

The study protocol was developed considering the SPIR IT checklist (Additional file 1) and guidelines and is registered in www.ClinicalTrials.gov (NCT04007705).

The study aims to analyze the effects of a potentially anti-inflammatory and low FODMAPs diet, compared to healthy eating recommendations, in disease assessment parameters, namely pain, fatigue, sleep quality, and GI alterations, in inflammatory markers and quality of life in FM patients.

Study design

A randomized controlled clinical trial, blind to patients, has started in April 2019 at the Portuguese Institute of Rheumatology [Instituto Português de Rematologia (IPR)] in Lisbon. All women diagnosed with FM followed-up at the IPR, with a medical appointment scheduled between February 2019 and December 2020, are being invited to participate in the study. The recruitment is being performed as the patients are identified in the appointment.

Study setting

After eligibility criteria confirmation and informed consent applied (Additional file 2), participants are being allocated to intervention or control group. Allocation of participants is performed using systematic procedures. Participants were sequentially assigned to intervention or control group as they were recruited. Due to the nature of the intervention, the allocation of experimental groups is blind to patients but not to researchers, as they will then apply the appropriate dietary plan. Each participant is given a code, to ensure anonymity and confidentiality of collected data.

Sample size

In order to define the sample size required for the study and to give a statistical power of 80%, G-Power Software version 3.1.9.4 revealed that, for a desirable effect size of 50%, a minimum sample size of 45 individuals is required. In order to prevent follow-up losses, the target sample size is $n = 100$.

Participant characteristics and eligibility criteria

Inclusion criteria are:

1- Female adults, aged over 18 and under 75 years old;
2- Diagnosis of FM performed by the physician, according to the Rome III criteria of the American College of Rheumatology, revised in 2010;
3- Ability to read and sign the informed consent; and

4- Stable dose therapy within 4 weeks before the study begins.

Exclusion criteria are:

1- Patients with pathologies that prevent to follow the dietary intervention;
2- Patients currently undergoing lactation or pregnancy;
3- Prior or current clinical history of abuse of drug or other substances;
4- Change of therapy during the intervention period;
5- Presence of other inflammatory diseases; and
6- Uncontrolled medical conditions (e.g., diabetes mellitus, heart disease, renal failure, neoplastic diseases, liver diseases).

Intervention

Patients are being contacted to schedule the first phase of the study (M0). During the first meeting with the researchers, a blood sample is collected, and the evaluation questionnaires are fulfilled. Additionally, anthropometric parameters (weight, height, and waist perimeter) and body composition are assessed, using a bio-impedance scale. The diet meal plan is determined by an investigator team nutritionist, according to the allocated group, taking into account basal metabolic rate, physical activity, lifestyle, food habits, and preferences of the patient, in order to ensure its feasibility. After 3 months of intervention, patients from both groups meet the researcher in order to perform a new blood collection, to assess weight and body composition and to complete all the evaluation questionnaires.

Dietary interventions specifications

The intervention group (G1) is adopting an anti-inflammatory diet, which is characterized by the exclusion of potentially inflammatory foods, namely gluten, dairy, sugar, and ultra-processed foods, over a consecutive period of 3 months. During the first month, a low FODMAPs diet is being implemented along with the anti-inflammatory diet, followed by the reintroduction of all fruits and vegetables over a consecutive period of 2 months, for a total of 3 months of intervention. The control group (G2) is adopting a diet based on recommendations for healthy eating in accordance with the World Health Organization (WHO) [30]. Both diets are determined by a nutritionist investigator during nutrition consultations using a leaflet, to help compliance. Examples of recipes are being delivered to help patients to comply with the outlined dietary plan. A table of foods to consume and to avoid is being provided to participants belonging to the intervention group during low FODMAPs diet phase (Additional file 3).

Adherence

During the intervention period, patients are being monitored every 15 days, by telephone, in order to assess compliance and any change regarding the inclusion and exclusion criteria, as well as to clarify any question about the intervention. Biweekly phone contacts are made in order to monitor the compliance with the recommendations. Participants are asked to fulfill a food diary of the 3 previous days to phone contact, and energy, macro, and micronutrients intake are then calculated for both groups. In addition, time of different meals is also analyzed. The Food Processor Software (version 11.2.274) is being used to analyze food records.

The experimental design of the present study is shown schematically in Figs. 1 and 2.

Intervention group

Anti-inflammatory diet

The anti-inflammatory diet is characterized by the exclusion of potential inflammatory foods, such as gluten, dairy, free sugar, and ultra-processed food, rich in sugar, hydrogenated fat, and food additives. Despite the controversy surrounding the ingestion of these foods, some authors defend the existence of an association of these with an increase in serum C-reactive protein (CRP) [18, 31, 32] and various inflammatory diseases [33], including rheumatic diseases [34].

Gluten Some authors describe an association between the characteristic symptoms of FM and the presence of altered intestinal permeability and dysbiosis [6, 7, 10]. In the presence of dysbiosis occurs the destruction of tight junctions, proteins present in enterocytes and responsible for preventing the entry of pathogens. The consequent intestinal hyperpermeability triggers, in turn, an immunological reaction of inflammatory character [35], described by several authors as low grade inflammation [36]. Intestinal hyperpermeability appears to be caused by several factors, including gliadin present in gluten [37–39]. In this way, it would be possible to suggest that the exclusion of gluten could allow a lower prevalence of dysbiosis, and therefore less intestinal inflammation.

Dairy There are several different casein subtypes in milk. In bovine milk, the predominant subtype is α-casein (50–55%), which does not exist in human milk [40], besides β-casein (35%) and κ-casein (15%). In addition, there are two types of β-casein, namely A1 and A2, being the A1 β-casein the prevalent one in Europe dairy products [41]. A systematic review concluded that A1 was associated to a higher prevalence of GI symptoms and increased intestinal inflammation in humans, compared to A2 [42]. The mechanism seems to be related to the activation of the Th2 signaling pathway in the intestine [43], which promote inflammation.

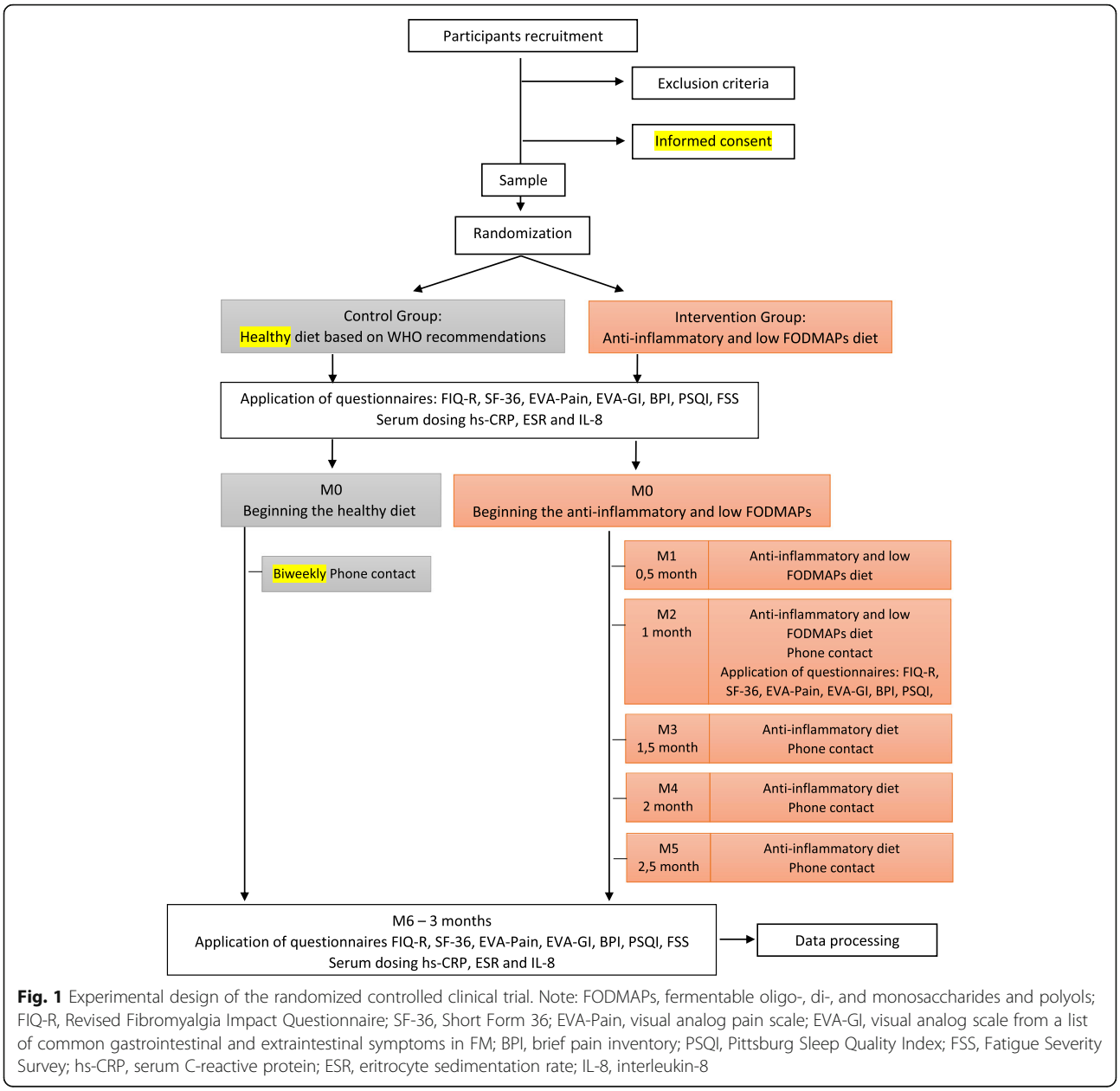

Fig. 1 Experimental design of the randomized controlled clinical trial. Note: FODMAPs, fermentable oligo-, di-, and monosaccharides and polyols; FIQ-R, Revised Fibromyalgia Impact Questionnaire; SF-36, Short Form 36; EVA-Pain, visual analog pain scale; EVA-GI, visual analog scale from a list of common gastrointestinal and extraintestinal symptoms in FM; BPI, brief pain inventory; PSQI, Pittsburg Sleep Quality Index; FSS, Fatigue Severity Survey; hs-CRP, serum C-reactive protein; ESR, eritrocyte sedimentation rate; IL-8, interleukin-8

Sugar Sugar is a recognizably inflammatory food. In recent years, WHO has been setting up standards for reducing its ingestion. Its excessive consumption promotes the production of free radicals, leading to an increase in oxidative stress [44]. On the other hand, a hyperinsulinogenic environment enhances the expression of pro-inflammatory molecules [45].

Ultra-processed foods Several authors define ultra-processed food as potentially inflammatory, mainly due to its free sugars, hydrogenated fat, and food additives content [46, 47]. Additionally, it is known that its relevant accumulation of advanced glication products (AGEs) is also related to a pro-inflammatory effect [48, 49]. When ingested, AGEs cross the epithelial barrier, attaching to the receptors in the dendritic cells of the mucosa, and promote the uptake of the antigens and to T cells, specifically Th1, Treg, Th2, and Th17, pro-inflammatory and inducers of allergic process [50]. AGEs in the cell activate cascades of signaling the production of inflammatory molecules, such as TNFα, IL6, and VCAM1 [51].

		STUDY PERIOD							
	Enrolment	Allocation	Post-allocation						Close-out
TIMEPOINT	*Feb-19 to Feb-2021*	Day 1	*Day 1*	*Day 15*	*Day 30*	*Day 45*	*Day 60*	*Day 75*	*Day 90*
ENROLMENT:									
Eligibility screen	X								
Informed consent	X								
Allocation		X							
INTERVENTIONS:									
Anti-inflammatory and Low FODMAPs diet			♦	—	—	—	—	♦	
Healthy Diet based on OMS recommendations (control)			♦	—	—	—	—	♦	
ASSESSMENTS:									
Questionnaires: FIQ-R, SF-36, EVA-Pain, EVA-GI, BPI, PSQI, FSS			X		X				X
Serum dosing hs-CRP, ESR and IL-8			X						X
Body Composition			X						X
3-day food records			X	X	X	X	X	X	X

Fig. 2 Schedule of enrolment, interventions, and assessments, according to SPIRIT guidelines. Note: FODMAPs, fermentable oligo-, di-, and monosaccharides and polyols; FIQ-R, Revised Fibromyalgia Impact Questionnaire; SF-36, Short Form 36; EVA-Pain, visual analog pain scale; EVA-GI, visual analogue scale from a list of common gastrointestinal and extraintestinal symptoms in FM; BPI, brief pain inventory; PSQI, Pittsburg Sleep Quality Index; FSS, Fatigue Severity Survey; hs-CRP, serum C-reactive protein; ESR, eritrocyte sedimentation rate; IL-8, interleukin-8

Anti-inflammatory food components There are some foods with recognized anti-inflammatory potential. Omega 3, especially at an adequate omega 6:omega 3 ratio, allows the production of prostaglandins, leukotrienes, resolvins, and protectins, which in turn promote the expression of anti-inflammatory cytokines [23]. In that sense, the ingestion of walnuts and omega 3 rich fish, such as tuna, mackerel, sardines, horse mackerel, and salmon, are being encouraged. Additionally, antioxidants in foods are known to decrease the free radicals production, which in turn helps to decrease the oxidative stress and, consequently, the expression of pro-inflammatory molecules [24, 52]. Thus, the intake of foods rich in antioxidants, such as fruit and vegetables, is also being promoted. Thus, the variability in the choice of vegetables and fruits was promoted, in order to obtain several different antioxidants, such as vitamin C (kiwi, orange), phenolic compounds (black grapes, pomegranate, blackberries, and raspberries), quercitin (apple), zeaxanthin (blueberries), indole-3-carbinol (broccoli, cabbage), and vitamin A (pumpkin, carrot, sweet potato). The intake of other foods rich in antioxidants was also promoted, such as cocoa, ginger, and white and green tea [53, 54].

Moreover, one of the most important factors in an anti-inflammatory diet is the maintenance of glycemic index, through a greater intake of fibers and suitable proteins and fats, against a balanced intake of carbohydrates.

Low FODMAPs diet

The presence of dysbiosis [4, 10, 12], and in particular of SIBO [8, 9], has been described in FM patients, with a significant improvement in pain, fatigue, gastric pain, mobility, and GI symptoms, after 4 weeks of low FODMAPs diet [11]. Marsh and colleagues meta-analysis support the efficacy of a diet with a low intake of foods rich in FODMAPs for a period of 4 to 6 weeks in the treatment of GI symptoms, including abdominal pain, abdominal distention, constipation, diarrhea, and flatulence [8], symptoms that are found very often in FM patients [4]. Since this is a recurrent situation in FM [4], it makes sense to start by trying to optimize the quality of the intestinal microbiota, in order to normalize these symptoms, before starting the anti-inflammatory diet.

This intervention involves avoiding all dairy; all cereals except rice and oats; cashew; all fruit other than banana, citrus, pineapple, red berries, strawberries, and kiwi; and all vegetables other than pumpkin, cabbage, lettuce, tomato, carrot, and cucumber, for a period of 4 to 6 weeks [8].

Control group

The control group is receiving a dietary meal plan based on healthy eating recommendations in accordance with WHO guidelines. According to WHO, a healthy diet contains at least 400 g of fruits and vegetables, excluding potatoes, sweet potatoes, cassava, and starchy roots. A consumption of legumes, nuts, and whole grains (wheat, maize, millet, oats, rice, rye) is also promoted, as well as an intake of less than 5 g of salt per day, less than 10% of total energy intake from free sugars and less than 30% of total energy intake from fats, giving preference to unsaturated fats [55].

Outcome measures

The primary PRO of interest for this study are pain, fatigue, quality of sleep, quality of life, GI symptoms, and the presence of inflammation. To determine the effect of dietary intervention on the disease, the following questionnaires are being included:

- Revised Fibromyalgia Impact Questionnaire (FIQR) [56], to verify the impact of FM on the patient's life;
- Visual analog pain scale (EVA_Pain) [57], validated by Boonstra and colleagues [58], and brief pain inventory (BPI) to assess pain [59], validated by Keller and colleagues [60];
- Visual analog scale from a list of common gastrointestinal and extraintestinal symptoms in FM, IBS, and non-celiac gluten sensitivity (NCGS) to assess GI symptoms [61], validated by Bengtsson and colleagues [62];
- Short Form 36 (SF-36) [63], to check the quality of life, validated by Fredheim and colleagues [64];
- Validated Fatigue Severity Survey (FSS) [65], to check the fatigue level;
- Validated Pittsburg Sleep Quality Index (PSQI) [66], to check the quality of sleep.

Additionally, serum high-sensitive CRP (hs-CRP), Erythrocyte Sedimentation Rate (ESR) and Interleukin-8 (IL-8) are being measured to assess the presence of inflammation. The serum collection and hs-CRP and ESR analysis is being perfomed by *Joaquim Chaves Saúde* Laboratory, an external entity. The biomarker IL-8 quantification is being performed according to ImmuliteR© (Siemens, Germany) manufacturer's protocol. Details on collection, laboratory evaluation, and storage of IL-8 is presented on Additional file 4.

Data on age, physical activity, and anthropometric parameters, such as waist circumference, height, and weight, are also being collected. Body composition, specifically fat mass, lean mass, and water, is being assessed by bio-impedance, through the scale of Inbody brand, model 770.

Patient and public involvement

Patients or members of the public were not involved in the design, conduct, reporting, or dissemination of the research.

Statistical analysis

Baseline demographic and clinical characteristics of the participants of both groups will be analyzed using descriptive statistics. For the continuous normal distributed variables, the t-student test will be used to assess the association between the disease evaluation parameters, the inflammatory markers, and the dietary intervention. Correlations between variables will be sought at the different assessment moments. Regression coefficients will be calculated to determine the contribution of the domains for each variable. ANOVA will be used to evaluate the participants' evolution within each group.

Missing data will not be included in the statistical analysis. Participants who discontinue or deviate from intervention protocols, as well as patients who meet exclusion criteria at some point of the intervention period, will be excluded. Motive of exclusion will be the outcome to be collected from these participants.

Statistical analysis will be performed using IBM SPSS Statistics Software, version 19.0. A p value of 0.05 is considered statistically significant.

Discussion

The results of this study are expected to determine whether a change in patient nutrition helps to alleviate symptoms, which would optimize medical intervention.

To our knowledge, a nutritional approach involving a combination of several anti-inflammatory dietary factors has never been designed. Nutritional approaches in FM, to date, had always isolated dietary components that are believed to have a negative effect on disease symptoms, such as the application of a gluten-free [67] and aspartame-free diet [68]. An integrative approach has never been undertaken to include anti-inflammatory components and exclude the pro-inflammatory ones.

A recent systematic review (2018) allowed us to determine that dietary interventions seem to be promising as complementary therapies in FM, particularly a hypocaloric diet [27, 28], a raw vegetarian diet [25, 26], or a low FODMAP diet [11]. However, the studies that exist are of poor quality, according to the Cochrane Risk of Bias [29]. In our study, we intend to increase the sample size and ensure a good completion of nutritional interventions, in order to increase the quality of the study.

The WHO recommendations in the control group, which already could have some positive impact on patients' health, could be a limiting factor in the interpretation of the results. However, once FM is associated with low-grade inflammation, those dietary recommendations per se may not be anti-inflammatory enough.

Trials status

This is the first trial Protocol version, submitted on July 13, 2020. The trial is currently ongoing. Recruitment started on April 9, 2019, and will end in February 2021. We expect the end of the study to take place by April 2021.

Abbreviations

AGE: Advanced glycated product; BPI: Brief pain inventory; GI: Gastrointestinal; ESR: Eritrocyte sedimentation rate; EVA-Pain: Visual analog pain scale; EVA-GI: Visual analog scale from a list of common gastrointestinal and extraintestinal symptoms in FM; FIQ-R: Revised Fibromyalgia Impact Questionnaire; FM: Fibromyalgia; FODMAPs: Fermentable oligo-, di-, and monosaccharides and polyols; FSS: Fatigue severity survey; hs-CRP: Serum C-reactive protein; IBS: Irritable bowel syndrome; IL: Interleukin; IPR: Instituto Português de Reumatologia; NSAID: Non-steroid anti-inflammatory drugs; PRO: Patient's reported outcomes; PSQI: Pittsburg sleep quality index; SF-36: Short Form 36; SIBO: Small intestinal bacterial overgrowth; WHO: World Health Organization

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13063-021-05146-3.

Additional file 1. SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Additional file 2. Informed Consent Statement.

Additional file 3. FODMAPs diet support table for patients.

Additional file 4. Interleukin-8 collection procedures.

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Not applicable.

Organizational structure and responsibilities

Trial Management Committee (TMC) members are ARS, JVP, MLS, and PP. Clinical Trial is monitored by the supervisors of the research work, namely JVP, MLS, and PP, for which periodic online meetings were held. Monitoring will be carried out during the implementation of the clinical trial, every 3 weeks.
Ethics Committee only evaluates the project at the initial moment.
Data monitoring committee (DMC) is not needed, given that the overall risk for patients is low, and that one of the authors is not blinded. Given the nature of the study, problems that are detrimental to the participant are not anticipated, and therefore there is no interim analysis.

Authors' contributions

ARS, AB, MFM, JVP, PM, MLS, and PP conceived and planned the study design. ARS is the sponsor investigator and is carrying out the intervention. The sponsor is responsible for the study design, execution of the protocol, collection, management, analysis, and interpretation of the data, writing the reports, and submission to publication. Within the implementation of the study, JVP is the doctor responsible for enrolling participants. The sponsor is assigning participants to group interventions, applying the dietary intervention, monitoring participants' adherence to diet, and collecting the data. JVP, MLS, and PP are currently helping to supervise the project. ARS wrote the first draft of the manuscript and all authors revised it critically for important intellectual content. All authors provided critical feedback and helped shape the research, analysis, and manuscript. The authors read and approved the final manuscript.

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Availability of data and materials

Anonymized dataset will be stored in Google Drive, available to all authors. Any data required to support the protocol can be supplied on request.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of both IPR and Cooperativa de Ensino Superior Egas Moniz, with reference number 4/2020, and is being carried out in accordance with the Declaration of Helsinki (Declaration of 1975, revised in 2000). An informed consent is being given to all participants, after oral and written information about the study. Each participant is given a code and the anonymity and confidentiality of the data collected is ensured.
There were no amendments to the trial protocol. However, any modifications to the protocol will be documented in a breach report form, the ethical committee notified, and subsequently, clinical trial registry updated.
Results will be submitted for publication in a peer-reviewed journal. The datasets of the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Competing interests

The authors report no financial or personal conflicts of interest.

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Publisher's Note

6.3. Manuscript III

Silva AR, Bernardo A, Mesquita MF, Vaz Patto J, Moreira, P, Padrão P, Silva, ML,

Dysbiosis, Small Intestinal Overgrowth, and Chronic Diseases: a translational approach

Treating Endocrine and Metabolic Disorders With Herbal Medicines, A. Hussain, and Shalini Behl, Editor. 2021, IGI Global. p. 334-362.

1. Introduction

Microbiota corresponds to the community of microorganisms that inhabit a specific environment of the human body. It can be found in skin, genito-urinay tract, mouth and intestine. Each microbiota is composed of bacteria that varies not only according to its environment, but also throughout individual's life. Regarding to human gastrointestinal tract, there are approximately 100 trillions of bacteria, classified according to phyla, classes, orders, families, genus and species. There are more than 1000 different species identified [1]. They are clustered in six phyla, namely *Firmicutes, Bacteroidetes, Actinobacteria, Verrucomicrobia, Proteobacteria* and *Fusobacteria.* Approximately 60% of the bacteria belong to the *Bacteroidetes* and *Firmicutes* phyla [2, 3]. For each of these phyla, there are several classes of bacteria.

Gut mucosa consists of an external intestinal barrier and an inner immunological barrier. Intestinal barrier is composed by commensal gut microbiota, mucous layer and intestinal monolayer. It is responsible for two fundamental functions for the individual's survivance: allowing nutrients absorption and defending the entry of foreign molecules to the organism. The inner layer barrier consists in immune cells organized in Gut-associated lymphoid tissue (GALT). GALT depends on the dendritic cells and the M-cells present in the Payer's patches to interact with luminal antigens [4]. The interaction between commensal bacteria and mucosal immune system is essential for immune function.

Integrity of these structures is necessary for maintenance of normal intestinal barrier function. The microbiota produces bacteriocins and short-chain fatty acids (SCFA), including butyrate, acetate and propionate, which inhibit the pathogenic growth of microorganisms; and defensins, which control bacteriocins and SCFA. On the other hand, the mucosal immune system produces immunoglobulin A (IgA), preventing pathogenic bacteria from entering in the epithelium [5].

There is a mutual benefit between the microbiota and host organism during homeostasis. While prebiotics ingested by the individual are the necessary substrate for its growth, bacteria provide maintenance of mucosal barrier integrity; synthesis of vitamins B (B1, B2, PP, biotin, pantothenic acid, folate, and B12) and K, amino acids, neurotransmitters (e.g. serotonin) and SCFA; promote a better absorption of other vitamins and minerals; promote lymphocyte maturation; and prevent entry of pathogens [5, 6].

After being produced by bacteria, SCFA are released in the intestinal lumen, quickly absorbed and used as energy mainly by colonocytes, specially butyrate. In its turn, acetate and propionate may be carried into the bloodstream and become available to a variety of different organs [7].

SCFA regulate countless processes, regarding to appetite and weight management; inflammatory responses from immune system; lipid oxidation; and thermogenesis in brown adipose tissue. In fact, butyrate is crucial to tissue barrier function, epigenetic regulation, immune-regulation, colonic integrity and homeostasis, intestinal transit and satiety [8]. During homeostasis, SCFA produce lactic acid and gases, namely carbon dioxide (CO_2), hydrogen (H^+) and methane (NH_4), which will lower the intestine pH and allowing the production of energy.

On the other hand, the deregulation of intestinal microbiota and SCFA production may be changed, and therefore the products of their metabolism will consequently be compromised. Inversely, the SCFA overproduction will promote an increase in concentration of lactic acid and gases causing flatulence and bloating [9]. Types and amounts of SCFA depend on the composition of microbiota.

2. Dysbiosis and Intestinal Permeability

Microbial programming begins in utero, and the composition of the microbiota is modulated by multiple factors including mode of delivery, gestational age, perinatal antibiotic exposure, feeding practices, environment, genetics, age, stress, diseases, and lifestyle, namely physical activity and diet quality and quantity [10]. Eating habits influence the gut bacterial structure and function during different time frames, including daily circadian rhythms of sleep-wakefulness and feeding-fasting cycle, and throughout the human lifespan [11].

A westernized dietary pattern rich in ultra-processed products, trans-fatty acids, sugars and refined flour, along with stress and physical inactivity, is known to be associated with changes in the intestinal microbiota [12, 13]. This promote alterations in the metabolism of bacteria and their overgrowth, with release of potentially toxic metabolites, such as endotoxins, hydrogen sulfide, phenols, ammonia and indoles. The intestinal mucosa is exposed to these metabolites, with harmful effects on the mucosa itself and host health [13].

Considering that changes in the microbiota composition are common, and its flexibility is considered normal, how can dysbiosis be defined? The critical differential factor is the host's response to changes in the microbiota composition. Dysbiosis is then a qualitative and quantitative change in the intestinal microbiota composition [14], in such a way that it induces an inflammatory response on the part of the host to the change in the microbiota composition compromising microbiota function. Dysbiosis lead to an increase of intestinal permeability, in which the intestine becomes more permeable to foreign and pathological agents [15, 16].

There is currently no specific biomarker to determine dysbiosis. However, in the last decades some alterations considered standard have been identified, in particular:

1- Reduction in overall microbial diversity of corresponding symbiotic community. Specifically, a depletion of obligate anaerobic bacteria such as *Bacteroides* and *Ruminococcus* spp., and conversely an increase in facultative anaerobes including *Enterobacteriaceae* (i.e. *E. Coli, Klebsiella* and *Proteus*) [17];

2- Preferential loss of organisms considered beneficial to human health and increase in *pathobionts*, i.e. members of the normal commensal microbiota, with the potential to cause pathology. This may translate in a reduction of *Firmicutes* and increase of *Proteobacteria* [18-20].

As a functional consequence of the loss of microbiota diversity, there appears to be a reduction in SCFA production, which compromises the metabolism stability.

2.1. Description and triggering factors

Increased intestinal permeability, defined by the destruction of tight-junctions and adherent-junctions, proteins that enable the enterocytes junction allowing the integrity of the intestinal mucosa, may arise as a result of dysbiosis. Its destruction increases the possibility of unwanted molecules entering the systemic circulation, including larger peptides, bacteria and lipopolysaccharides (LPS), triggering a pathological inflammation that harms the immune system. If prolonged over time, it can lead to the development of autoimmune diseases and immunodeficiency [15, 21, 22]. Intestinal hyperpermeability may be triggered by ingestion of gliadin [23], alcohol [24, 25], increased bile acids concentration [26], zinc deficit [27], vitamin D deficit [28] and non-steroid antiinflammatoy drugs (NSAIDs) [29].

Regarding gliadin, a gluten constituent protein present in wheat, rye and barley, it is particularly related to the recognition of LPS by the TLR4, which causes consequent activation of the Nuclear Factor kappa B (NFkB) signalling pathway, promoter of inflammatory cytokine expression [23], namely Tumor Necrosis Factor Alpha (TNF-α) and Interleukine-1 (IL-1) [30, 31]. Increased intestinal permeability caused by gliadin can be identified by increased serum zonulin, a protein whose expression appears to be activated by gliadin, which binds to Protease Activated Receptor 2 (PAR2) and Epithelial Growth Factor Membrane Receptors (EGFR), inducing the destruction of tight-junctions [32].

Changes in barrier function are also related to an increase in TNF-α, IL-1β and IL-13, expressed in chronic low-grade intestinal inflammation [33]. Measurements over 0.3 mg/dL of serum ultra-sensitive Reactive C Protein (usCRP) reveals low-grade inflammation [34, 35].

Chronic inflammation appears to result from an inadequate immune response as a result of genetic predisposition, as well as changes in the intestinal microbiota. On the other hand, an insufficient response to a stimulus of a bacterium results in an insufficient immune response to pathogens [36].

2.2. Influence of food and nutrients on dysbiosis

Single food components, salt, food additives, pre- and probiotics, and different dietary patterns may change the composition of the intestinal microbiota [4]. Human intestine microbiota plasticity can respond efficiently and rapidly to external variable, as confirmed by changes in the microbiota composition detected within 24 hours, in a clinical trial that compared high-fat low-fiber and low-fat high-fiber controlled diets [37]. However, short- and long-term dietary interventions differently impact the intestinal microbiota composition [38, 39].

Combining information from two reviews [4, 36], although further double-blind human intervention studies are still needed, there is already enough information to indicate that there are some modulating dietary factors in the composition of the intestinal microbiota.

2.2.1. Carbohydrates and gut microbiota

Carbohydrates can be categorized as digestible and non-digestible molecules. Digestible carbohydrates are enzymatically degraded and released as glucose in bloodstream. Indigestible carbohydrates are resistant starch and dietary fibers, which could be fermentable in colon and soluble in water, or insoluble and non-fermentable. Prebiotics are fermentable dietary fibers, that allow better development and activity of bacteria in the intestinal microbiota, especially in the colon [40].

Beneficial effect of prebiotics on health has been widely recognized [41-43], particularly oligosaccharides, such as fructooligosaccharides (FOS) and inulin, present in bananas, onions, garlic, leeks, asparagus, chicory, yacon potatoes; gel-forming fibers, such as guar gum and

psyllium husk; beta-glucan present in oat; and the resistant starch present in the green banana [44]. Several studies have pointed out the effectiveness of increased prebiotics intake in changes in intestinal microbiota composition. Several animal models and humans trials with inflammatory bowel disease (IBD) have reported that supplementation of some types of dietary fibre can prolong remission and reduce lesions of the intestinal mucosa during the progression of the disease [45]. Additionally, a study points to a reduction in *Firmicutes* and an increase in *Bacteroides* with a diet rich in prebiotics, which in turn improve glucose sensitivity, inflammation and oxidative stress [46].

2.2.2. Proteins in gut microbiota

Fermentation of amino acids occurs in distal colon mainly by *Firmicutes, Bacteroides* and *Proteobacteria*.

Animal-based protein, particularly from red meat and dairy products, may lead to an increase of bile tolerant anaerobic bacteria, such as *Bacteroides, Alistipes* and *Bilophila*, which promotes an increase in Trimethylamine N-oxyde (TMAO) [47], associated with increased risk for cardiovascular disease [48, 49]. In fact, proteolitic excessive fermentation produces a decrease in SCFA, and an increase in potentially toxic substrates, such as ammonia, nitrosamines and TMAO. Additionally, the intake of animal protein is also associated with an increase in hydrogen-sulfide by sulfate-reducing bacteria, and a decrease in *Bifidobacterium* [50], which increases the risk of IBD.

On the other hand, the intake of plant-based protein seems to have a beneficial impact on the intestinal microbiota. Humans clinical trials where pea protein has been used have promoted an increase in commensal *Bifidobacterium* and *Lactobacillus*, and a decrease in pathogenic *Bacteroides fragilis* and *Clorstridium perfringens* [51]. In fact, vegetarians and vegan microbiota composition differs from omnivores. Some studies showed higher ratio of *Bacteroides/Prevotella, along with higher occurrence of Bacteroides thetaiotaomicron, Clostridium clostridioforme, Klebsiella pneumoniae, and Faecalibacterium prausnitzii* and low occurrence of *Clostridium cluster* and *Bilophila wadsworthia* in vegetarians and vegans [4, 52, 53]. However, the effects of phenolic compounds should be taken into account, as these components increase the abundance of beneficial bacteria such as *Bifidobacterium and Lactobacillus*.

2.2.3. Lipids in gut microbiota

Lipids can be characterized in three classes: saturated, monounsaturated and polyunsaturated fatty acids. Saturated fatty acids are found mainly in animal fats, such as meat and dairy products. High fat diet, specially saturated fat, is associated to dysbiosis. In western diets, the intake of saturated fat is particularly high, and associated with a reduced intake of fiber. Diets high in saturated fat and in low fiber contribute to metabolic endotoxemia [54]**.** High saturated fat diet stimulates production of sulphate-reducing bacteria, such as *Bilophila wadsworthia*. These bacteria may reduce dissulfide bonds in mucus, causing alteration in mucus layer stability and consequent inflammation [55, 56].

Monounsaturated fatty acids (MUFA) are found in olive oil, olives and avocado. A systematic review showed that high MUFA diet has no effect on microbiota composition, distribution or *Bacteroides-Firmicutes* ratio. However, MUFA were positively correlated with *Parabacteroides*, *Provetella*, and *Enterobacteriaceae* family, and low *Bifidobacterium genus* [57].

Polyunsaturated fatty acids (PUFA) include n-3 and n-6 families. The n-3 PUFA are found in some fish, such as sardines, mackerel and salmon, in nuts, flaxseeds and sea algae, such as kelp. The n-6 PUFA are found in sunflower, corn and soybean oil.

The n-3 PUFA exert a beneficial effect in intestine, by restoring *Firmicutes-Bacteroides* ratio and increasing *Lachnospiraceae* family, both associated to increase of butyrate SCFA [58]. In the past, the n-6:n-3 ratio has enjoyed widespread use and was set at and ideal value of 1:1; however, this metric has both theoretical and practical difficulties, and is now outmoded [59]. Nevertheless, in most industrialized countries with a westernized diet, this ratio is sometimes used to describe values between 10:1 and 50:1, which correlate to increased risk to cardiovascular and chronic disease [60], increased intestinal permeability and metabolic endotoxemia [61]. In this sense, it is essential to promote a greater intake of n-3 PUFA, considering simultaneously a delicate balance with n-6 PUFA.

2.2.4. Vitamins and Minerals in gut microbiota

Some vitamins can be synthesized by the intestinal microbiota, namely thiamine, riboflavin, niacin, biotin, pantothenic acid, folate, cobalamin and vitamin K. *Bacteroidetes, Fusobacteria and Proteobacteria* are primarily responsible for the synthesis of these vitamins [4].

Additionally, some micronutrients are essential for intestinal health. Zinc may contribute to the host defence by maintaining the membrane barrier. An *in vitro* study where zinc deprivation was induced, revealed a disruption of membrane barrier integrity that led to an upregulation of chemokines, which plays a role in neutrophil migration and inflammatory development. It was seen an increase in the migration of neutrophils and secretion of IL-8, epithelial neutrophil activating peptide-78, and growth-regulated oncogene-a, alterations that were not found when culture medium was replete with zinc [27]. Several researchers point to zinc therapeutic effect, through the maintenance of the enterocytic barrier [62]. Therefore, its adequate nutritional supply must be taken into account.

Iron is another mineral with an important impact on the intestine, whose availability influences microbiota composition. Constante *et al.* demonstrated in mice that a heme-rich diet decreased microbiota diversity, having promoted an increase in the concentration of *Proteobacteria* and decreased *Firmicutes* [4].

Regarding vitamin D, there has been increase evidence of its antibacterial effect. Vitamin D induces the expression of cathelicidin antimicrobial peptide (CAMP) gene, which plays a critical role in innate immune defence and enhances barrier function [63]. In experimental studies, Kong *et al.* demonstrated that activated vitamin D, 1,25-dihydroxy vitamin D (1,25(OH)2D3) increase tight junction (TJ) proteins, zonula occludens and E-cadherin [64], which suggest its importance in maintenance of the mucosal barrier. Jin *et al*. suggested that vitamin D receptors (VDR) status could influence the mice intestinal microbiota both taxonomic and functional levels. The authors advocated that VDR is crucial for the maintenance of microbial homeostasis. In humans, a reduction in the production of 1,25(OH)2D3 or in the expression of VDR may lead to gut inflammation and an increase in *Proteobacteria* colonization. This leads to an alteration in the balance of the microbiota composition, inducing dysbiosis [65]. Additionally, in IBD patients, vitamin D has a recognized positive effect, by modulating the gut microbiome and increasing the abundance of potentially beneficial bacterial strains [66].

2.2.5. Redox activity in gut microbiota

Several studies demonstrate the antioxidants effects in gut microbiota composition. In the carotenoid family, lutein significantly promotes the growth of *Bifidobacteria* and *Lactobacillus*, and a decrease in *Bacteroides* and *Clostridium*, in humans [67]. On the other hand, quercetin supplementation significantly improved the *Firmicutes-Bacteroides* ratio, and inhibited the growth of bacteria associated with obesity, such as *Erysipelotrichaceae, Bacillus* spp. and *Eubacterium cylindroides*, in mice fed with high-sugar high-fat diet [68]. Also anthocyanines, which have a known anti-inflammatory effect against colorectal cancer significantly stimulates growth of *Bifidubacterium* spp., *Lactobacillus* and *Enterococcus* spp. [69].

However, a study suggested that the anti-inflammatory effects of beta-carotene were mediated by the gut microbiota [70]. Also with regard to phenolic compounds, a similar effect occurs, as the intestinal microbiota is able to modulate probiotic activity and influence its bioavailability [71]. These facts suggest that an intestinal dysbiosis environment may remove less nutrient absorption and interfere with its antioxidant and anti-inflammatory activity.

2.2.6. Food additives in gut microbiota

Ultra-processed foods frequently have emulsifiers in its composition, such as lecithins and mono- and diglycerides of fatty acids. These molecules may increase bacterial translocation across epithelial, promoting systemic inflammation and altering microbiota composition [72]. Emulsifiers intake is associated to a decrease in diversity of microbiota composition, a decrease in *Bacteroides* and an increase in *Verrumicrobia*, specifically *Akkermansia muciniphila* and *Proteobacteria*, leading to dysbiosis and chronic gut inflammation [72, 73].

Regarding to non-caloric artificial sweeteners, a systematic review showed an alteration in gut microbiota composition after ingestion of these molecules, particularly in respect to saccharin and aspartame [74]. Although data is scarce, studies have found similar results, both in animal (mice) and human models. After ingesting 50-100mg of sodium saccharin (NaS), there was an increase in the number of anaerobic bacteria, namely *Bacteroides and Clostridiales*, and a decrease in *Lactobacilus* [74]. A cohort study conducted by Suez and colleagues found a positive correlation between NaS and central obesity, Hemoglobin A1C and impaired glucose tolerance in 381 non-obese individuals who reported regular consumption of artificial sweeteners. One hundred and seventy-one randomly selected individuals showed intestinal microbiota changes, particularly an increase in *Enterobacteriaceae, Deltaproteobacteria and Actinobacteria phylum [75]*. Regarding to steviol glycosides, there are no reported consistent microbial changes [74].

Another molecule present in westernized countries diet, are designated advanced glycation end-products (AGEs). AGEs form during heating and processing of food products. It is vastly known its impact on increasing risk for chronic diseases [76], inflammation [77], oxidative stress and insulin resistance [78]. Additionally, limiting AGE intake may lead to a decrease in inflammation and chronic diseases related to inflammatory status [77]. In peritoneal dialysis patients, dietary AGE restriction altered the bacterial gut microbiota with a significant reduction in *Prevotella copri* and *Bifidobacterium animalis* and increased *Alistipes indistinctus*, *Clostridium citroniae*, *Clostridium hathewayi*, and *Ruminococcus gauvreauii* relative abundance [79]. However, there is conflicting evidence regarding the impact of dietary AGEs on gut microbiota reshaping [80].

2.2.7. Dietary patterns in gut microbiota

If nutrients and bioactive food molecules influences the composition of the intestinal microbiota, then the differences in dietary patterns will have to manifest themselves significantly in the intestine. Table 1 illustrates the effect of different dietary patterns on microbiota and health.

Table 1: Dietary pattern, microbiota composition and health consequences.

Diet	Microbiota composition	Molecular and metabolic modifications	Health consequences
Vegan / Vegetarian diet	↓ Bifidobacteria ↑ Clostridium clostridioforme ↓ Clostridium cluster XIV ↑ Klebsiella pneumoniae ↓ Bilophila ↑ Bacteroides/Prevotella ↑ Bacteroidetes	Unkown	Unkown
Mediterranean diet	↑ Bifidobacteria ↑ Lactobacillus ↓ Clostridium ↑ Lachnospiraceae ↓ Enterobacteria ↑ Bacteroidetes	↑ SCFA production ↑ Microbiota diversity and stability ↑ Antiinflammatory citokine expression (IL10, IL22)	Prevention of metabolic diseases [81]

Western diet	↓ Bifidobacteria ↑ Ruminococcus torques ↓ Roseburia ↓ Eubacterium rectale ↓ Ruminococcus bromii ↓ Lactobacillus ↑ Enterobacteria ↑ Bilophila ↑ Alistipes ↓ Prevotella ↑ Bacteroides ↑ Akkermansia	↓ SCFA production ↓ Microbiota diversity ↑ Profinflammatory citokine expression (IL17, TNFα, IFNγ) ↑ Endotoxins, hidrogen sulfide, phenols, ammonia, índoles	Increased risk of metabolic diseases (obesity; cardiovascular disease; diabetes mellitus type II) [82]
Low FODMAPs diet	↓ Bifidobacteria ↓ Ruminococcus gravus ↓ Clostridium ↓ F. prausnitzii ↓ Akkermansia	↓ Microbiota diversity and abundance	<u>If applied for over 6 weeks period:</u> Possible weight loss [83]; Deficit of antioxidants (flavonoids, carotenoids, vitamin C, phenolic acid and anthocyanins) [84]

Legend: SCFA – Short Chain Fatty Acids; FODMAPs – Fermentable oligo-, di- and monosaccharides and polyols; IL - Interleukine

The composition of the microbiome of modern civilizations with different lifestyles mimics the evolution between bacteria and the human host [39]. In a study carried out by Quercia and colleagues, six population groups, namely from Hazda, Malawi, Burkina Faso, Italy (adults and children) and the USA, were investigated with regard to their lifestyle and eating habits and composition of the intestinal microbiota. USA and Italy follow a western diet, based on farinaceous, refined sugar, saturated and trans fat, and high meat consumption, and have a sedentary and stressful lifestyle. Burkina Faso and Malawi inhabitants have a traditional rural African diet that is rich in starch, fibers, and plant foods. Hadza is a tribe from Tanzania, whose lifestyle remains the same as that of their ancestors, eating game meat, tubers, fruits and berries. Investigators identified a great variety in the composition of the microbiota between the various communities. Specifically, there was a higher abundance of *Ruminococcaceae* distinguishing for the Hadza hunter-gatherers, the emergence of *Clostridiales* and *Prevotella* in

rural Malawi and Burkina Faso populations, and the dominance of the *Faecalibacterium* in Western populations [39, 85].

Other study compared the intestinal microbiota composition of European individuals with the one from Burkina Faso individuals, where the diet is based on millet, local vegetables and a low intake of animal fat and protein. It was found that individuals from Burkina Faso had a higher concentration of *Provetella* and *Xilanibacter*, and a decrease in *Proteobacteria,* compared to European individuals [86].

Intestinal microbiota composition of individuals from Venezuela, Malawi and United States was compared. It was found that, regardless of age, the composition of the microbiota of individuals from Venezuela and Malawi was similar. Individuals from United States showed less diversity of intestinal microbiota, with a reduction in *Provetella* and an increase in bile tolerant bacteria such as *Alistipes, Bilophila* and *Bacteroides*, and a decrease in *Firmicutes* [87].

These differences come from diets composition of these populations. Westernized diet is rich in saturated fat, sugar, refined flours, food additives and AGEs, and low in antioxidant compounds, fibers and n-3 PUFA. This diet lead to an increase in bacteria of *Clostridium innocuum*, *Catenibacterium mitsuokai* and *Enterococcus*, and a decrease in *Bifidobacteria* spp. The increase in the ingestion of n-6 PUFA from sunflower oil, also common in westernized diet, promotes the reduction of *Firmicutes*, and the increase of *Actinobacteria* and *Proteobacteria*. On the other hand, the consumption of whole grains and fibers is associated with an increase in *Bifidobacteria longum, Bifidobacteria breve* and *Bifidobacteria theyaiotaomicron*, and a decrease in *Mycobacterium* and *Enterobacteriaceae* [12].

In the Mediterranean diet, whose concept was created to mimic the food of the inhabitants of Greece, Crete and southern Italy in the 1960s, the consumption of fruit, vegetables, olive oil, nuts, whole grains and fish is promoted. Thus, this diet translates into a high intake of fiber, antioxidants, PUFA and MUFA, being low in saturated fat, sugar and food additives. These characteristics improved *Firmicutes-Bacteroides* ratio and increased *Bifidobacterium* and SCFA production [88-90].

In addition to the dietary aspects, it is important to remember that there are other factors that negatively influence the intestinal microbiota composition, such as physical inactivity, chronic stress, abuse of antibiotics and exposure to xenobiotics, such as tobacco and pollution.

2.2.8. Probiotics in gut microbiota

According to Food and Agriculture Organization (FAO), probiotics are defined as live microorganisms that, when administered in the adequate amounts, exert health benefits on the host [91]. Probiotics act to restore microbial balance, optimizing its metabolic, protective and structural functions [92]. Some examples are yogurt, kefir and kombucha. Additionally, it can be taken as a supplement, in which case they must present a significant phyla diversity, especially *Firmicutes* and *Bacteroides* [93-98].

The effectiveness of probiotics is proven for a wide variety of pathologies. In IBD, the use of probiotics has been extensively studied, with several meta-analyses that affirm its effectiveness, especially in ulcerative colitis (UC) [99-101].

The effect of probiotic supplementation alone on Helicobacter Pylori (H. Pylori) eradication are minimal, although they suggest a direct and positive role [102]. Nevertheless, the use of probiotics has been suggested as an adjunct to the usual medical therapy for the treatment of H. Pylori, with very interesting results not only with regard to the effectiveness of the antibiotic in the complete eradication of the bacteria, but also in the replacement of the intestinal microbiota [102].

In respect to oral health, literature suggests that probiotics usage could be beneficial due to its ability to decrease the colony forming units counts of the oral pathogens. However, randomized clinical trials with long-term follow-up periods are needed to confirm their efficacy in reducing the prevalence/incidence of oral infectious diseases [103]. Additionally, other systematic reviews demonstrate the beneficial effects of probiotics in Non-Alcoholic Fat Liver Disease (NAFLD) [104] and neurological diseases like Depression [105].

Regarding safety of probiotic usage, some studies indicate that some adverse effects may arise, particularly sepsis, fungemia and gastrointestinal ischemia. These effects are usually one-off and mostly in critically ill patients in intensive care units, critically sick infants, post-operative and hospitalized patients and patients with immune-compromised complexity [106]. Some authors advocate taking prebiotics and probiotics in a combined way, thus taking advantage of the synergy between them created [40].

2.3. Dysbiosis in Chronic Diseases

Dysbiosis seems to be associated to systemic and chronic metabolic diseases. However, the mechanism by which dysbiosis and the progression of chronic diseases are related remains

unclear. There are two probable situations that occur in a very common way, and that, individually or in combination, can explain this connection: low-grade inflammation and bacterial translocation.

Many authors describe the presence of low-grade inflammation in many different chronic diseases, like rheumatoid arthritis, cystic fibrosis, psoriasis, periodontitis, diabetes mellitus type 2 and obesity. The inflammatory process develops in the presence of an inflammatory stimulus, such as trauma or infection. Local macrophages are activated, which produce IL-1β and TNF-α. These two cytokines bind to endothelial cell receptors, inducing the inflammatory response. At the molecular level, within the macrophage, transcription factors bind to DNA promoting the expression of pro-inflammatory molecules. In case of NFkB signalling pathway, this protein is found outside the nucleus inhibited by IkBα. The inflammatory stimulus leads to an increase in kinases that destroy IkBα, releasing NFkB, which binds to DNA and increases the inflammatory cytokine outflow. These cytokines, namely IL-1β, IL-6 and TNF-α, are released into endothelial cells, where in addition to promoting local inflammation, they will destroy endothelial IkBα, releasing NFkB to express more proteins, such as Thelper (Th) 1, Th2 and Th17, and Tregulators (Treg) lymphocytes, selectins, Vascular cell adhesion protein 1 (VCAM-1) and Ciclo-oxigenase-2 (COX-2), amplifying the inflammatory response [107, 108]. The more extensive or systemic the inflammatory stimulus, the greater the production of pro-inflammatory molecules [108].

COX-2, being responsible for the metabolism of arachidonic acid, promotes an increase in Prostaglandines E_2 (PGE_2), which in turn increases intestinal permeability [109]. Enterocytes themselves increase the production of PGE_2, so that intestinal hyperpermeability allows macrophages to enter and carry out its process. However, this same increase in permeability allows the occurrence of bacterial translocation and/or bacterial products, such as LPS, peptidoglycans, muramyl-dipeptides and bacterial DNA. This mechanism occurs across gut mucosal barrier to mesenteric lymph nodes, liver, spleen, kidney and bloodstream [110], which justifies the manifestation of dysbiosis in numerous chronic diseases.

The association of dysbiosis with IBD including UC and chron's disease (CD), has been demonstrated [111, 112]. Several authors describe that bacterial alterations in the composition of intestinal microbiota strongly correlate with disease status [113-116].

However, there are other chronic conditions whose patients have changes in the intestinal microbiota composition such as, metabolic syndrome [12, 111], diabetes mellitus type 2[117], atherosclerosis [118] and obesity [117]. The difference in gut microbiota of obese and non-obese individuals have been vastly reported [119]. In fact, a western diet has been shown to decrease

beneficial bacteria [50]. From a sociological and behavioural perspective, some authors suggest that SCFA would have a protective role against obesity, since they regulate hormones related to appetite control as previously mentioned, such as peptide YY (PYY), glucagon-Like peptide-1 (GLP-1) and leptin. In the presence of dysbiosis, the production of SCFA may be reduced, and as a consequence there may be a disturbance in the regulation of these hormones, further aggravating the individual's behaviour in an attempt to control his disease [120].

Several rheumatological diseases, namely ankilosing spondylitis [121], systemic sclerosis, rheumatoid arthritis [122], psoriasis and fibromyalgia (FM), present alterations in the composition of the intestinal microbiota. In a study by Malatji and colleagues, several metabolites were identified in the urine of FM patients, by Hydrogen nuclear magnetic resonance spectroscopy (^{1}H NMR), suggesting changes in the intestinal microbiota, namely: 1) hyperuric acid, increased in the presence of reflux or hepatic detoxification; 2) 2-hydroxyisobutyrate acid, associated with the presence of *Faecalibacterium Prausnitzii*, a commensal bacteria; 3) lactic acid. On the other hand, they also identified taurine, succinate acid and TMAO as being the responsible metabolites for differentiation between patients of this pathology and the control group, also indicators of intestinal microbiome alteration [123]. Additionally, some therapeutic strategies developed with the aim of normalizing the microbiota have shown positive results [124]. In patients with Cystic Fibrosis, taking probiotics showed an improvement in respiratory function [125]. However, effectiveness is still too limited to realize their application in the clinic.

Finally, the relationship between the brain and the intestine is already well documented. Many hormones and peptides produced in the intestine, such as PYY, and others such as leptin, ghrelin and insulin, can influence neurological function. The Brain Derived Neurotrophic Factor (BDNF) produced in the brain, modulates metabolic functions such as appetite suppression and insulin sensitivity. [126]. Several authors report an alteration of the intestinal microbiota and the presence of dysbiosis in patients with neuropsychiatric diseases and central nervous system (CNS) disruption [127, 128], such as depression [129], schizophrenia [130], attention-deficit hypersensitivity disorder [131] and autism spectrum disorders [132].

3. Small Intestinal Bacterial Overgrowth

During homeostasis, microorganisms are distributed throughout the entire gastrointestinal tract, from the mouth to the anus. This distribution varies qualitatively and quantitatively in each environment. In the stomach, duodenum and proximal jejunum are found between 10^1 and 10^3 colonyforming units (CFU) of bacteria per mL; in distal jejunum and ileum between 10^4 and 10^7 CFU/mL; and in the colon between 10^{11} and 10^{12} CFU/mL [133]. In the presence of dysbiosis, one of three situations may occur: a migration of bacteria from the colon to the duodenum and proximal jejunum; an excessive proliferation of bacteria already present in the duodenum; or the appearance and subsequent proliferation of a nefarious bacteria in this region of the intestine [134]. If we find ourselves in this scenario, we will probably be at the Small Intestine Bacterial Overgrowth (SIBO) demonstration.

3.1. Description and triggering factors

SIBO is defined as an increase in the number and/or alteration in the type of bacteria in the upper gastrointestinal tract [134]. This situation leads to an alteration in intestinal track motility, often reflected in diarrhoea, constipation or to an alternation between the two. In addition to these symptoms, a variety of clinical complaints such as abdominal pain, bloating, flatulence, lack of energy and weight loss, are also common [135]. As a result, the nutrients absorption can be compromised. The activity of brush border enzymes disaccharidase and hydrolase will be inhibited, which leads to a decrease in digestion and absorption of carbohydrates. In addition, the deconjugation of bile acids by bacteria may results in malabsorption of fat and liposoluble vitamins, such as vitamin A, D and E [134, 136]. In contrast, levels of vitamin K, a fat-soluble vitamin, are usually normal [136]. Vitamin B12 deficiency may result from inhibition of normal B12 absorption by anaerobic organisms, and by the consumption of this vitamin within the intestinal lumen by enteric facultative microbes before it could be absorbed. Iron and vitamin B1 and B3 deficiencies have also been described in the setting of SIBO, although the mechanisms are not known [134, 136].

Prevention of bacterial overgrowth is possible through several endogenous defence mechanisms of our organism, namely gastric acid secretion, intestinal motility, intact ileocecal valve, immunoglobulins within intestinal secretion and bacteriostatic properties of pancreatic and biliary secretion. Besides dysbiosis caused by an inadequate life style, aetiology of SIBO is associated with disorders of protective antibacterial mechanisms (e.g. immunodeficiency syndromes), imbalances in gastrointestinal enzyme production (e.g. achlorhydria, pancreatic

exocrine insufficiency), anatomical abnormalities (e.g. small intestinal obstruction, diverticulosis, development of fistulae, surgical blind loop, loss of competence of the ileocecal valve) and/or motility disorders (e.g. scleroderma, autonomic neuropathy in diabetes mellitus, post-radiation enteropathy, small intestinal pseudo-obstruction). In some patients more than one factor may be involved [134].

3.2. SIBO in Chronic Diseases

The assessment of prevalence of SIBO in chronic diseases is difficult to do, mainly because tests used for the diagnosis of SIBO vary considerably in the still few studies carried out. However, the studies and systematic reviews that exist, despite their limitations, reveal significant results for the prevalence of SIBO in several chronic diseases.

Obesity could be a predisposing factor for SIBO, and several studies suggest an increased risk of developing SIBO in obese individuals compared to non-obese individuals. A meta-analysis found that the risk of SIBO was almost two times higher among individuals with obesity compared to individuals without obesity, however there was no statistical significance. Nevertheless, the risk increased to threefold and reached statistical significance when only studies from Western countries were included [137]. Authors suggest two possible mechanisms to explain this observation. Firstly, the increased risk of gut dysmotility seen in individuals with obesity. Obesity negatively affects bowel motility by markedly increasing the occurrence of clustered contractions in the small intestine, which consequently could affect propulsive motility and therefore affect the bacteria natural life cycle, resulting in accumulation of bacteria [138]. This phenomenon, also seen in other pathologies, such as cirrhosis, portal hypertension, pancreatitis and IBD, have shown similar disruptions in the Migrating Motor Complex (MMC), resulting in the ineffective sweeping of bacteria from the proximal bowel into the colon [139]. The second explanation is related to alteration of gut microbiota and consequent dysbiosis, which has been seen in obese patients [137, 140].

IBD is associated with physiological phenomena of alteration of enzyme activity, loss of intestinal mucosa integrity and the presence of dysbiosis, which makes patients with this pathology more predisposed to the development of SIBO. A systematic review with meta-analysis identified a statistically significant prevalence of SIBO of 22.3% ($p<0.05$) in patients with IBD, 14.3% ($p<0.05$) in patients with UC and 25.4% ($p<0.05$) in patients with CD [141], which is corroborates within another systematic review [101]. It was also found that loss of ileocecal valve (due to previous

ileocecal resection) and/or large entero-enteric and enterocolic fistulae are important predisposing factors in IBD [101, 134, 141].

The prevalence of SIBO in patients with Irritable Bowel Syndrome (IBS) is 38% ($p<0.05$) [142], which is specially significant in this population.

With regard to Celiac Disease, although some authors point to the presence of SIBO in some patients [134], a meta-analysis that there is no significant relationship [143].

Regarding Chronic Liver Disease (CLD), there is a consistent and statistically significant increase of SIBO in patients with the disease, with an Odds Ratio (OR) for SIBO in CLD of 7.15% ($p<0.05$). Particularly, in Non-Alcoholic Fat Liver Disease (NAFLD), the prevalence of SIBO is 33.5% ($p<0.05$), comparing to healthy control (7.3%, $p<0.05$). Studies have shown that patients with NAFLD and SIBO had significant higher blood endotoxin concentration compared with control. Additionally, TLR expression and serum TNFα and IL-8, which correlate with TRL-4 expression, were significantly higher in these patients. SIBO-associated increased intestinal permeability and endotoxemia results in activation of TLR signalling, that plays an important role in NAFLD and progression to Non-Alcoholic Steatohepatitis (NASH). On its turn, in Cirrhosis, the prevalence of SIBO is 40.1% ($p<0.05$), comparing to healthy control (7.3%, $p<0.05$) [144].

Chronic Pancreatitis (CP) is characterized by inflammatory and destructive functional changes in pancreas. There are some predictor factors to SIBO development, such as fat malabsorption, diabetic neuropathy, proton pump inhibitor (PPI) drugs use, alcohol intake and surgical procedures. A meta-analysis reveals an OR for SIBO in CP of 4.1 ($p<0.05$) [145], which suggest a significant prevalence of SIBO in these patients. In respect to Systemic Sclerosis (SSc), the prevalence of SIBO range 30 to 62% [146]. Other studies associate the persistence of the FM symptoms with the presence of SIBO [147-149], in particular the intensity of pain, as SIBO appears to increase the exposure of immune system cells to antigens in the intestinal lumen, thereby causing immune modulation [123].

Additionally, SIBO seems to be present in others manifestations, such as dyspepsia, rosacea, restless legs syndrome, hypothyroidism, Parkinson's disease, diabetes, coronary artery disease, and abdominal surgery (e.g., hysterectomy, gastrectomy, cholecystectomy, and colectomy). However, the prevalence of SIBO in patients with these associated conditions is highly variable, with a range between 4% and 79% [150].

3.3 Influence of food and nutrients on SIBO

Conventional therapy involves the prescription of antibiotics, usually broad-spectrum Rifaximin, often applied 8-8h for 7 days. However, antibiotic therapy is not associated with a complete improvement in clinical symptoms, which leads to the very common need to repeat the prescription after 1 month [101]. Additionally, the association of the antibiotic use and abuse with development of dysbiosis is well known [151, 152]. This often result in intolerance to treatment, *Clostridium dificile* infection and increase in antibiotic resistance [101, 136, 153, 154].

Other strategy for SIBO treatment includes the introduction of probiotics. A meta-analysis verified that probiotics supplementation could effectively decontaminate SIBO, decrease H_2 concentration, and relieve abdominal pain, but were ineffective in preventing SIBO [155]. However, it has been verified that the intervention of probiotics can lead to the opposite result of the expected, with the exacerbation of the symptoms. It is possible that the effectiveness of using probiotics depends on the type of bacteria present in the product, and whether or not they are combined with prebiotics. Many probiotics on the market contain FOS, which are saccharides more fermentable by bacteria and that could therefore cause a worsening of symptoms. However the composition of the supplements used is not specified.

The intervention must always be individualized. Nutritional support is essential, mainly due to the possibility of nutritional deficits that SIBO entails [134]. One of the most used nutritional approaches is the application of a diet low in fermentable oligo-, di- and monosaccharides and polyols (FODMAPs) foods.

Low FODMAPs Diet is a two-phase diet, characterized by avoidance of slowly absorbed or nondigestible short-chain carbohydrates (i.e. FODMAPs) for a period of between 4 and 6 weeks, followed by a slow reintroduction of well tolerated food. FODMAPs are a large class saccharides mainly absorbed in the colon, forming H_2 and CH_4 as a consequence of its metabolism by bacteria. The total daily intake of FODMAPs in a habitual diet ranges from 15 grams to 30 grams per day [84]. However, in the presence of an overgrowth of bacteria in the duodenum and proximal jejunum, this metabolism will generate flatulence, bloating and abdominal pain, classic SIBO symptoms [147]. Table 2 show the food alterative for Low FODMAPs diet.

Table 2 - Food alternatives poor in FODMAPs - adapted from Hill et. al., 2017 [156].

FODMAPs	**Foods high in FODMAPs**	**Suitable alternatives low in FODMAPs**
Excess of Fructose	Fruits: apple, peach, mango, pear, pea, watermelon, preserves	Fruits: banana, melon, grape, grapefruit, melon, kiwi, lemon,

	Honey sweeteners: fructose, corn syrup Large total dose of fructose: concentrated sources of fruit, large portions of fruit, dried fruit, fruit juice	lime, orange, passion fruit, papaya, raspberry, blueberry, strawberry, pineapple Honey substitutes: maple syrup Sweeteners: any sweeteners, except polyols
Lactose	Milk: regular and low-fat cow, goat, and sheep milk; ice cream Yogurts: regular and low-fat yogurts Cheeses: soft and fresh cheeses	Milk: lactose-free milk, rice milk Ice cream substitutes: gelato, sorbet Yogurts: lactose-free yogurts Cheeses: hard cheeses
Oligosaccharides (fructans and/or galactans)	Vegetables: artichoke, asparagus, beet, broccoli, Brussels sprouts, cabbage, fennel, garlic, leeks, okra, onion, pea, shallot Cereals: rye and wheat cereals (for example, biscuit, bread, couscous, biscuit, pasta) Legumes: baked beans, chickpeas, lentils, red beans Fruit: watermelon	Vegetables: bamboo root, spinach, carrot, celery, pak choy cabbage, cucumber, chives, corn, eggplant, green beans, lettuce, pumpkin, chard Cereals: bread / cereals gluten-free and spelled products Fruit: tomato
Polyols	Fruits: apple, apricot, avocado, cherry, lychee, nectarine, peach, pear, plum, watermelon Vegetables: cauliflower, mushroom, pea Sweeteners: isomalt, maltitol, mannitol, sorbitol, xylitol, and other sweeteners ending in "-ol"	Fruits: banana, blueberry, melon, grape, grapefruit, melon, kiwi, lemon, lime, orange, passion fruit, papaya, raspberry Sweeteners: glucose, sugar (sucrose), other artificial sweeteners that do not end in "-ol"

The FODMAPs mechanism of action is linked to the stimulation of mechanoreceptors as a response to luminal distension from a combination of increased luminal water content from the osmotic effect, especially in the small intestine, and from the release of H_2 and NH_4 from the bacterial fermentation of saccharides [156]. Such stimulation can lead to ascending messages that might be interpreted as abdominal pain or bloating; reflex responses to the diaphragm and anterior abdominal wall, leading to increased abdominal distension; and effects on motility with potential change in bowel habits [84, 156]. Additionally, there could occur an excessive production of SCFA, which could lead to visceral sensitivity and high-amplitude propagated colonic contractions, thus accelerating intestinal transit [84].

In this context, limiting the intake of the most fermentable carbohydrates will potentially alleviate the symptoms, by reducing the formation of gases. There is still insufficient evidence to consider Low FODMAPs Diet a legitimate first-line therapy, mainly because most of the

studies carried out are of low quality, with short durations, small number of patients and inappropriate comparator placebo groups [156].

A positive effect of Low FODMAPs Diet in gastrointestinal manifestations, specially in IBS has been suggested. IBS patients are probably the population where more clinical trials have been performed on a diet low in FODMAPs. In a randomized placebo-controlled trial, 104 IBS patients carried out a Low FODMAPs Diet or a placebo diet for four weeks, similar in amount of food restriction and in difficulty of implementation. Patients on the Low FODMAPs Diet had a significantly symptom relief (61%, $p < 0.05$) and a significant improvement in the results of the disease assessment questionnaire Irritable Bowel Syndrome Severity Scoring System (IBS-SSS) compared to placebo ($p < 0.001$) [157]. Additionally, a meta-analysis showed a significant improvement in the quality of life questionnaires (IBS-QOL) and in IBS-SSS, as well as in symptoms such as bloating and abdominal pain, supports the efficacy of a low FODMAP diet in the treatment of functional gastrointestinal symptoms [147].

Although there are no studies carried out with the application of Low FODMAPs Diet in other pathologies, the presence of SIBO in diseases such as NAFLD, CP and SSc, among others, suggests that this intervention could be beneficial in these patients. In fact, a four week Low FODMAPs Diet clinical trial implemented in 38 FM patients showed a significant improvement in pain, fatigue, gastric pain, mobility and gastrointestinal symptoms [158].

The composition of the intestinal microbiota is sensitive to several aspects, not only with regard to dietary habits but also general lifestyle components. The association of dysbiosis is the promotion of low-grade inflammation and the development of chronic diseases is, as we have seen, a reality.

4. References

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6.4. Manuscript IV

Silva AR, Bernardo MA, Mesquita, MF, Vaz Patto J, Moreira, P, Silva, ML, Padrão, P

Effect of an anti-inflammatory and low Fermentable oligo-, di- and monosaccharides, alcohol and polyols (FODMAPs) diet in fibromyalgia: a randomized controlled trial.

[submitted for publication]

An anti-inflammatory and low fermentable oligo-, di- and monosaccharides and polyols (FODMAPs) diet improved patient reported outcomes in Fibromyalgia: a randomized controlled trial

Running title: An anti-inflammatory diet in Fibromyalgia

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ABSTRACT

Background: Fibromyalgia (FM) has been associated with dysbiosis and low-grade inflammation. Studies have reported that diet influence clinical features in FM.

Objective: To evaluate the effect of an anti-inflammatory and low fermentable oligo, di- and monosaccharides and polyols (FODMAP) diet in clinical outcomes of FM patients.

Methods: This Randomized Controlled Trial (NCT04007705) included 46 FM female patients. Intervention group (n=22) adopted an anti-inflammatory diet for 3 months, excluding gluten, dairy, added sugar and ultraprocessed foods, along with a low FODMAPs diet in the first month. Control group (n=24) followed general healthy eating recommendations. Before and after intervention, participants were assessed regarding pain, fatigue, gastrointestinal symptoms, quality-of-sleep and quality-of-life, through: Revised Fibromyalgia Impact Questionnaire (FIQR), Visual Analogue Pain Scale (VAS), Visual Analogue Scale from gastrointestinal symptoms (VAS GI), Brief Pain Inventory (BPI), Pittsburg Sleep Quality Index (PSQI), Fatigue Severity Survey (FSS) and The Short Form Health Survey (SF-36). A blood sample was collected and High-sensitive C-Reactive Protein and Erythrocyte Sedimentation Rate were quantified. Paired Samples T-Test/Wilcoxon and independent samples T-Test/Mann-Whitney were used to compare variables between groups.

Results: After intervention, there was an improvement in intervention group scores of FIQR (p=0.001), VAS (p=0.002), BPI (p=0.011), FSS (p=0.042), VAS_GI (p=0.002), PSQI (p=0.048), and SF36 (p=0.045) compared to control group. Inflammatory biomarkers (hs-CRP, ESR) did not change in both groups. The intervention was beneficial in the intervention group, regardless of age, disease duration, body mass index variation and body fat changes between baseline and post-intervention.

Conclusion: An anti-inflammatory and low-FODMAP diet improved clinical features in FM patients and may be useful as a complement to pharmacological therapy.

KEYWORDS

Fibromyalgia

Inflammation

Anti-inflammatory diet

Low FODMAPs diet

SIBO

Dysbiosis

Randomized Controlled Trial

INTRODUCTION

Fibromyalgia (FM) is a chronic non-degenerative disease, characterized by generalized chronic musculoskeletal pain, fatigue, asthenia, anxiety, depression, changes in sleep pattern and gastrointestinal symptoms similar to Irritable Bowel Syndrome (IBS) [1].

FM pathophysiology is still not known. However, low-grade inflammation is described by several authors, through a plasma pro-inflammatory cytokines increase, particularly interleukin (IL)-6 and IL-8 [2, 3]. Literature suggests that saturated fatty acids (SFA), *trans* fatty acids and cholesterol intake, included in the "Dietary Inflammatory Index" [4], together with gluten [5], dairy products [6] and ultra-processed foods [7], could have a pro-inflammatory effect. On the other hand, it is known the anti-inflammatory potential of mono- and poly-unsaturated fatty acids (PUFA) [4], specially omega-3 [8], and antioxidants compounds in the diet [9].

Furthermore, several studies showed an association between FM and dysbiosis [10], and in particular with Small Intestinal Bacterial Overgrowth (SIBO) [11, 12], characterized by the inappropriate colonization of the distal small bowel with colonic bacteria [12]. SIBO is usually treated with a 4 week low fermentable oligo, di- and monosaccharides and polyols (FODMAPs) diet protocol [11].

As pharmacological therapy seems not to completely resolve the symptoms of the disease [1], a dietary intervention which includes potentially anti-inflammatory foods and excludes the potentially pro-inflammatory ones, and that simultaneously allows an optimization of the intestinal microbiota, emerges as an opportunity to improve the FM patient's reported outcomes (PRO). Therefore, the aim of this study was to evaluate the effects of a potentially anti-inflammatory and low FODMAPs diet in clinical features, namely pain, fatigue, sleep quality, gastrointestinal alterations and inflammatory biomarkers of FM patients.

MATERIALS AND METHODS

The detailed study protocol of this Randomized Controlled Clinical Trial (RCT) has been published elsewhere [27] and registered in Clinicaltrials.gov with the identification number: NCT04007705.

Ethical Considerations

This study was approved by the Ethics Committee of Portuguese Institute of Rheumatology, with reference number 4/2020, and was carried out in accordance with the Declaration of Helsinki (Declaration of 1975, revised in 2000). An informed consent was given to all participants, after oral and written information about the study.

Study Design and participants

This parallel-group RCT with two arms, blind to patients, took place between April 2019 and June 2020 at the Portuguese Institute of Rheumatology (*Instituto Português de Reumatologia*) in Lisbon, Portugal.

Forty-six female adults, aged between 18 and 75 years old, which were not currently undergoing lactation or pregnancy, and with ability to read and sign the Informed Consent were eligible to integrate the study. FM diagnose has been performed by a Rheumatologist, according to the Rome III criteria of the American College of Rheumatology, revised in 2010 [13], with a stable dose therapy within 4 weeks before the study beginning.

Patients with the presence of other inflammatory diseases or uncontrolled medical conditions (e.g. Diabetes Mellitus, heart disease, renal failure, neoplastic diseases, liver diseases), with prior or current clinical history of abuse of drug or other substances, or with diagnose of any pathologies that prevent to follow the dietary intervention identified by the physician were not included. Patients which changed pharmacological therapy during the intervention period were excluded.

After eligibility criteria confirmation and informed consent signed, participants were randomly allocated to intervention or control group. The first patient was randomly assigned to intervention (G1) or control group (G2), and the following patients were systematically allocated to each group, as they were recruited. Each participant was given a code and anonymity and confidentiality of the collected data was assured.

Sixty-two patients were assessed for eligibility, 61 were included and 46 completed the study (Figure 1). At baseline, the participants' mean age was 57 years. The general characteristics for the participants are shown in Table 1. For 3 months, intervention group adopted a two phases intervention: the first phase, occurred in the first month, in which an anti-inflammatory diet and low FODMAPS diet was adopted; the second phase occurred in the second and third subsequent months, and participants continued only with the anti-inflammatory diet. Control group adopted a healthy diet, based on the World Health Organization (WHO) general recommendations [14]. Patients' reported outcomes (PRO) were collected by interview using structured validated questionnaires, and a blood sample was taken for the measurement of serum inflammatory biomarkers, before and after intervention. Patients were monitored through biweekly telephone contacts, being also possible for the patient to clarify any question through the contact provided.

Dietary implementation

Intervention group

Intervention group adopted an anti-inflammatory diet, excluding potential inflammatory components/foods, such as gluten, dairy products, free sugars, and ultra-processed food. Furthermore, the ingestion of foods rich in omega-3 fatty acids, antioxidants and dietary fiber was promoted, according the "Dietary Inflammatory Index" [4, 15]. During the first month of intervention, a low FODMAPs diet criteria has been added to the anti-inflammatory diet, with the exclusion of foods rich in sugars more fermentable by bacteria. After the first month of intervention, all fruit and vegetables previously excluded were reintroduced, keeping the anti-

inflammatory diet for another 2 months, completing a total of 3 months of intervention. A trained registered dietitian provided recipes in order to help diet compliance.

Anti-inflammatory diet. The anti-inflammatory diet combined the exclusion of potentially pro-inflammatory components and the inclusion of potentially anti-inflammatory ones.

Gliadin, present in gluten, is one of the known causes of intestinal hyperpermeability, which triggers an immunological reaction of inflammatory character [16], described by several authors as low-grade inflammation [17]. Dairy were excluded considering the variation of beta-casein genotypes in milk and their possible association with gastrointestinal symptoms [18] and increased intestinal inflammation through activation of the Th2 signaling pathway in the intestine [19]. Sugar has a recognized inflammatory activity, as its excessive consumption promotes the production of free radicals, leading to an increase in oxidative stress [20]. Moreover, a hyperglycemic and hyperinsulinogenic environment enhances the expression of pro-inflammatory molecules [21]. Many ultra-processed foods are considered potentially inflammatory due to its free sugars, hydrogenated fat and food additives content [22, 23]. Additionally, it is known that its relevant accumulation of Advanced Glication End-products (AGEs) is also related to a pro-inflammatory effect [24, 25], by promoting TNFα, IL6, VCAM1, Th1, Treg, Th2 and Th17 liberation, which induce inflammation [26, 27].

On the other hand, to increase antioxidant and anti-inflammatory potential, the ingestion of 3 pieces of fruit a day and half a plate of vegetables twice a day was promoted. The intake of red fruits, strawberries, pomegranates, red grapes, apple (rich in flavenols, such as resveratrol and quercitin), orange, kiwi, papaya (rich in vitamin C) was indicated. The intake of indole-3-carbinol and sulforaphanes present in broccoli, cauliflower and cabbage was promoted, with the indication of cooking for a maximum of 5 minutes to preserve it. It was also promoted the increased intake of beta-carotene rich foods (carrots, pumpkins, orange sweet potatoes),

lycopene (tomatoes, blueberries), gingerol (ginger) and catechins (cocoa and green tea) [28]. Moreover, it is well known the omega-3 anti-inflammatory capacity, especially at an adequate omega-6:omega-3 ratio. It allows the production of prostaglandins, leukotrienes, resolvins and protectins, promoting the expression of anti-inflammatory cytokines [15, 29]. Therefore, the consumption of omega-3 rich food such as salmon, tuna, mackerel and sardines, as well as walnuts, almonds and linseeds, was promoted. Furthermore, the replacement of sunflower oil, butter and margarines for extra virgin olive oil was also indicated, for an increase of monounsaturated fatty acids and reduction in omega-6 and saturated fat. Additionally, the maintenance of glycemic index was promoted, through an adequate intake of dietary fiber, protein and fat, and a balanced intake of carbohydrates, since is one of the most important factors in an anti-inflammatory diet.

Low FODMAPs diet. The low FODMAPs diet is characterized by the avoidance of all dairies; all cereals except rice and oat; cashew; all fruits other than banana, citrus, pineapple, red berries, strawberries and kiwi; and all vegetables other than pumpkin, cabbage, lettuce, tomato, carrot and cucumber.

The presence of dysbiosis [30-32], and in particular of SIBO [11, 12] has been described in FM patients, with a significant improvement in pain, fatigue, gastric pain, mobility and gastrointestinal symptoms, after 4 weeks of low FODMAPs diet [33]. Marsh and colleagues meta-analysis support the efficacy of a diet with a low intake of foods rich in FODMAPs for a period of 4 to 6 weeks in the treatment of gastrointestinal symptoms, including abdominal pain, abdominal distention, constipation, diarrhea and flatulence [11].

Control group

The control group adopted healthy eating WHO recommendations which were explained to participants. According to WHO, a healthy diet contains at least 400g of fruits and vegetables, excluding potatoes, sweet potatoes, cassava and starchy roots. A consumption of legumes, nuts and whole grains (wheat, maize, millet, oats, rice, rye), was also promoted, as well as an intake of less than 5 g of salt per day, less than 10% of total energy intake from free sugars and less than 30% of total energy intake from fats, giving preference to unsaturated fats [14].

Socio-demographic and life-style characteristics assessment

Socio-demographic characteristics of the patients were collected, namely age, education level (< 9 schooling years or ≥ 9 schooling years) and work status (employed, unemployed, retired or domestic/pensioner).

Life-style characteristics, such as smoking habits (recoded as smoker or non-smoker), frequency of alcohol beverages intake (recoded as daily or occasionally, since only one participant reported a regular consumption) and structured physical exercise (< 1 hour a week or ≥ 1 hour a week), were collected. Additionally, it was also registered the disease duration and usual pharmacological therapy.

Anthropometric and body composition assessment

Data on anthropometric measurements namely waist circumference, height and weight were assessed at beginning and in the end of the intervention. Body mass index (BMI) (kg/m^2) was calculated, and WHO classification was used to categorize BMI [34].

Body composition parameters namely fat mass percentage, muscular mass and total body water were estimated by bio-impedance, through the scale Inbody ®, model 770.

Post-intervention and baseline difference was arithmetic calculated for each anthropometric and body composition variables.

Patient reported outcomes

The primary PRO of interest for this study were pain, fatigue, quality of sleep, quality of life and gastrointestinal symptoms, which were assessed through specific questionnaires.

Revised Fibromyalgia Impact Questionnaire (FIQR) [35], was used to assess the impact of FM on the patient's life. It consists of 21 questions that evaluate clinical severity, health status and ability to daily activities of FM patients. A score between 0 and 100 is obtained, which is lower as the quality of life improves.

Visual Analogue Pain Scale (VAS) [36] and Brief Pain Inventory (BPI) were used to assess pain [37]. VAS is a one item questionnaire about pain, which score range is between 0 and 10, being 0 equivalent to no pain and 10 the worst pain ever felt. BPI measures pain intensity and pain interference in daily activities. The score ranges between 0 and 20, being lower as lower pain is felt.

To assess gastrointestinal symptoms, Visual Analog Scale from a list of common gastrointestinal and extraintestinal symptoms in FM, IBS and Non-Celiac Gluten Sensitivity (VAS_GI) [38] was applied. VS_GI score was between 0 and 10, being 0 equivalent to very good gastrointestinal function and 10 very bad gastrointestinal function.

Fatigue Severity Survey (FSS) [39] was used to assess the fatigue level. This tool is a 9 items questionnaire which evaluate motor aspects of fatigue and its impact on individual’s daily functioning. The scale ranges from 0 to 7 and reveals less fatigue the lower the score obtained.

Pittsburg Sleep Quality Index (PSQI) [40] was used to assess the quality of sleep. This questionnaire evaluates subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, use of sleeping pills and daytime dysfunction. PSQI score range is between 0 and 21. A total score above 5 indicates poor sleep quality.

To assess quality of life, Short Form 36 (SF-36) [41] was used. SF-36 is a 36 items tool that focus general health, physical functioning, vitality, physical pain, mental health, social functioning, and emotional impact on daily tasks. Score range is between 0 and 100, being 100 equivalent to the better possible quality of life. It encompasses both Mental and Physical Health that were quantified separately, in addition to the whole questionnaire.

Biochemical parameters assessment

A blood sample was collected at baseline and post-intervention. Blood tests were carried out by analysts from *Joaquim Chaves Saúde* Laboratory, at Portuguese Institute of Rheumatology. Serum high-sensitive C-Reactive Protein (hs-CRP) and Erythrocyte Sedimentation Rate (ESR) were measured through immunoturbidimetry [42] and Westergren method [43] respectively, to assess the presence of inflammation. Despite being both nonspecific markers, the combination of the two allows obtaining information on the individual's inflammatory phenotype. Being an acute phase protein, CRP reveals the presence of inflammation in its initial phase, increasing after 4-6 hours. On the other hand, the ESR increases within 24-48h and gradually decreases, allowing to assess the response to a treatment [44].

Dietary and nutritional assessment

At baseline, a 24-hour dietary recall was applied to verify the homogeneity on dietary intake between groups. Every biweekly telephone contact and at the end of the intervention, a 3-day food record was completed by each participant in order to ensure the intervention compliance. Study participants were carefully instructed by a dietitian to complete the food record. If necessary, participants estimated the food amounts with pictures book which estimate the portion sizes for meals [45].

The Food Processor ® software version 11.2.274 was used to convert food into nutrients. Energy and nutrients were expressed by average values calculated from the 3-day food records. Protein, carbohydrates, of which sugars, monosaccharides, disaccharides and added sugars, total fat, of which MUFA, PUFA, omega-3 and omega-6 were expressed by percentage of TEI (% TEI). Dietary fiber was expressed in grams and g/1000kcal

Additionally, the average of the 3-day food record of the ingested amount of food containing gluten in its composition (bread, biscuits, cake, pasta, savoury, breakfast cereals, cereal bars) was manually collected from food diaries and 24 hours report. The same foods in the gluten-free version were not considered. Moreover, dairy products (milk, yogurt, cheese, butter), ultra-processed products according to the NOVA classification system [46], and sugar added to beverages were also collected and expressed in grams.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics Software, version 19.0.

Descriptive data were presented as mean, standard deviation (SD), median, percentile (P) 25 and P75 for continuous variables or the frequency (number and percentage) for categorical variables.

To compare FM symptoms and inflammatory biomarkers within-groups at baseline and post-intervention, Paired Samples T-Test or Wilcoxon signed rank test were used for continuous variables, as appropriate.

Independent Samples T-Test or Mann-Whitney U test were used to compare FM symptoms, inflammatory biomarkers and dietary intake between groups at baseline and at post-intervention moments, as appropriate. The arithmetic differences between baseline and post-intervention were calculated for dietary intake and clinical features for each group. MANOVA was applied to assess the effect of the intervention between groups.

Additionally, a General Linear Model (GLM) was used in order to assess the impact of the intervention adjusting for potentially confounders, namely age, disease duration, variation of BMI and variation of body fat percentage. GLM was also used to verify the possible isolated effect of the each nutrient and food with anti-inflammatory potential in the clinical features.

In order to define the sample size required for the study and to give a statistical power of 80%, G-Power Software version 3.1.9.4 revealed that, for a desirable effect size of 50%, a minimum sample size of 45 individuals was required.

RESULTS

Baseline characteristics of the participants

The study sample consisted of 62 adult female FM patients of which 46 patients completed the study. There were no significant differences between intervention group (n = 22) and control group (n = 24) for demographics, life-style characteristics and body composition (Table 1).

Almost 40% of the participants were employed and had less than 9 schooling years. More than 85% reported being non-smoker, more than 91% did not drink alcoholic beverages daily and more than 91% exercised less than 1 hour a week. Both groups had a body fat mass average of 39%, and BMI of nearly 30 kg/m^2.

Regarding usual pharmacological treatment, over than 50% in both groups were medicated with analgesics and muscle relaxants, and approximately 75% reported to take antidepressants, anxiolytics, or sedatives.

Dietary and nutritional data

At baseline, no significant differences were observed between groups in most of the nutritional parameters, except for the intake of total energy and omega-3 fatty acids, and for

the consumption of added sugars and ultra-processed products which were significantly higher in control group (Table 2).

The control group maintained dietary intake, with no differences between baseline and post intervention. However, intervention group reported significant changes after the implementation of the dietary protocol, with a negative variation in the contribution to TEI for protein (-2.1 ± 4.2 % to TEI, p=0.03), carbohydrates (-5.9 ± 9.9 % to TEI, p=0.011), sugars (-7.5 ± 9.1 % to TEI, p=0.001), disaccharides (-3.3 ± 3.0 % to TEI, p<0.001) and SFA (-3.0 ± 4.1 % to TEI, p=0.006). On the contrary, a positive variation was found for total fat (9.4 ± 9.8 % to TEI, p=0.001), PUFA (5.0 ± 10.1 % to TEI, p=0.022), omega-3 fatty acids (0.7 ± 0.046), and fibre/1000kcal (0.4 ± 0.9 % to TEI, p=0.037). Additionally, intervention group reported the exclusion of sugar added to foods (baseline 1.1 ± 3.7 g; post-intervention 0 g, p<0.001) and ultra-processed foods (baseline 47.3 ± 44.1 g; post-intervention 0 g, p<0.001), as prescribed.

Despite the statistically similar baseline values, there were significant differences between intervention and control group in the post-intervention period regarding the intake of disaccharides, added sugar and SFA, which was higher in control group, and concerning the intake of total fat and PUFA that was higher in intervention group (Supplementary Table 1).

Fibromyalgia clinical features

The differences between post-intervention and baseline showed significantly more favourable outcomes for the majority of parameters in intervention group compared to control group. Significantly greater improvement was found in FM severity scale FIQR in intervention group compared to control group (-19.9 ± 18.8 vs -2.2 ± 16.1; p=0.001). Significantly greater improvement was found in pain in intervention group compared to control group, both in VAS (-2.3 ± 2.5 vs -0.04 ± 2.1; p=0.002) and BPI questionnaires (-3.8 ± 4.1 vs -1.1 ± 2.6; p=0.011. Significantly greater improvement was found in gastrointestinal symptoms, through VAS_GI

questionnaire, in intervention group compared to control group (-2.0 ± 0.9 vs -0.9 ± 1.3; p=0.002). Significantly greater improvement was found in sleep quality, in PSQI questionnaire, in intervention group compared to control group (-3.5 ± 4.6 vs -1.2 ± 2.6; p=0.048). Significantly greater improvement was found in fatigue, through FSS questionnaire, in intervention group compared to control group (-1.1 ± 1.2 vs -0.5 ± 1.0; p=0.042). Significantly greater improvement was found in quality of life, evaluated through SF36, in intervention group compared to control group (10.2 ± 11.2 vs 3.6 ± 10.4; p=0.045), specifically in physical component (18.1 ± 20.0 vs 3.9 ± 13.5; p=0.008). SF36 score is higher as quality of life improves (Table 2 and 3).

At baseline, the between-group analysis showed no differences for the majority of parameters evaluated except for BPI, FSS and SF36, for which the intervention group had more favourable baseline values.

In respect to intervention group, there was observed an improvement between baseline and post-intervention in FIQR (59.3 ± 9.2 vs 39.5 ± 21.8; p<0.001), in VAS (7.7 ± 1.4 vs 5.4 ± 2.3; p=0.001), BPI (12.5 ± 2.3 vs 8.7 ± 4.7; p<0.001), FSS (5.5 ± 1.1 vs 4.4 ± 1.7; p=0.001), VAS_GI (3.4 ± 1.5 vs 1.4 ± 1.3; p<0.001), PSQI (15.0 ± 5.2 vs 11.6 ± 5.7; p=0.002), SF36 (44.0 ± 10.3 vs 54.3 ± 12.3; p<0.001); SF36 physical component (33.4 ± 11.4 vs 51.5 ± 18.8; p<0.001) and SF36 mental component (54.4 ± 23.1 vs 63.4 ± 21.4; p=0.023).

In control group, there was also found an improvement in VAS_GI (3.1 ± 1.4 vs 2.3 ± 1.3; p=0.007), FSS (6.4 ± 0.7 vs 5.9 ± 1.2; p=0.038) and PSQI (15.1 ± 4.0 vs 13.9 ± 4.5; p=0.037) at the end of intervention compared to baseline.

Inflammatory biomarkers (hs-CRP, ESR) did not significantly change in both groups (Table 4).

With regard to weight status and body composition, it was found that, in the control group, there were no differences between baseline and post-intervention (BMI: 29.5 ± 5.8 vs 29.2 ± 5.5;

p=0.078; body fat percentage: 39.1 ± 8.9 vs 37.7 ± 10.9; *p*=0.181). However, in the intervention group there were significant changes between the two moments, both in BMI (28.6 ± 4.1 vs 27.6 ± 3.9, *p*>0.001) and body fat percentage (38.5 ± 6.4 vs 37.0 ± 7.0; *p*=0.015).

It was possible to observe that, the impact of the intervention on FM symptoms was beneficial in the intervention group regardless of age, disease duration, BMI variation and body fat mass variation between baseline and post-intervention. When the impact of the variation in the intake of each nutrient per se (monosaccharides, disaccharides, dietary fiber, omega 3 fatty acids and omega 6 fatty acids) on FM clinical features was tested, there were no significant differences between post-intervention and baseline moments.

The effect of the intervention between groups remain significant for FIQR, VAS and VAS_GI after a multivariate analysis.

DISCUSSION

After the anti-inflammatory and low FODMAPs nutritional intervention, there was an improvement in FM symptoms, namely pain, fatigue, gastrointestinal symptoms, quality-of-sleep and quality-of-life in intervention group.

Our results are aligned with other dietary interventions. An aspartame-free diet [47], a vegetarian diet [48, 49] and a hypocaloric diet [50, 51] reduced pain in FM patients. Also, Marum and colleagues found that a 4 weeks low FODMAPs diet reduced pain and improved quality of life in FM patients [33]. However, the nutritional interventions carried out so far were of poor statistical quality, according to a recent systematic review [52]. Additionally, every study carried out so far tested the effect of isolated dietary strategies. In the present study, we used an integrative nutritional and dietary approach, which included anti-inflammatory components and excluded the pro-inflammatory ones, therefore promoting more consistent results. In fact, the absence of individual significant nutritional predictors, namely

monosaccharides, disaccharides, dietary fiber, omega-3 fatty acids and omega-6 fatty acids, reflects that the interventions with a reductionist nutritional approach, focusing on single nutritional factors may not be enough to improve FM symptoms. Instead, our results provide a novel dietary intervention approach that combines nutritional and dietary strategies with anti-inflammatory potential. Several authors defend that the effect of the overall diet or a dietary pattern appears to have more impact in chronic diseases risk than looking for isolated nutrients [4, 53]. To the best of our knowledge, this is the first study that brings together the multiplicity of food characteristics and nutritional factors with plausibility to improve FM symptoms.

In addition, our study considered a wide variety of outcomes, assessed through validated instruments, in order to broaden the ability to assess typical FM symptoms. We consider this aspect of great importance, given the broad spectrum of symptoms characteristic of the disease, and the absence of specific instruments for its assessment.

It has been reported that weight loss was the main reason for pain improvement in FM patients in dietary interventions [54]. However, in this study we showed that the improvement in FM symptoms after intervention was independent of body fat mass percentage variation and BMI variation between baseline and post-intervention. This fact suggests that a hypocaloric diet and weight management may not be enough to improve FM symptoms.

Although the FM pathophysiology is not known, it has been suggested that genetic predisposition and stressful life events may trigger central and peripheral nervous system mechanisms [55], which is related to neuro-inflammation. The central nervous system (CNS) activation, associated with an apparent dysfunction in ascending and descending neural pathways in these patients, lead to an increased response mediated by amplification of CNS signalling. On the other hand, peripheral nervous system (PNS) is responsible for activation of mediators of innate immunity, promoting the release of bradykinin, histamine, serotonin, tumor necrosis factor (TNF), cytokines and IL, which translate inflammatory response and neuro-

inflammation [56]. In this context, the anti-inflammatory nutritional approach employed in the present study may have contributed to reduce the systemic inflammatory process present in FM, and could provide an explanation of the mechanisms behind our findings. We also suggest that anti-inflammatory dietary intervention could also allow a more attenuated immune response, with a possible decrease in IL and pro-inflammatory cytokines. Although its alteration has already been detected in FM patients [57, 58], CRP and ESR biomarkers, which were used in our study, may not be specific enough, and that could possibly be the reason why there were no differences in our study between baseline and post-intervention.

Additionally, some authors revealed an association between FM and intestinal inflammation [32, 59, 60], derived from an alteration of the intestinal microbiota, with consequent intestinal dysbiosis and SIBO [11, 12, 61]. Dysbiosis and metabolic endotoxemia are associated with a westernized dietary pattern rich in ultra-processed products, trans-fatty acids, sugars and refined flour, along with stress and physical inactivity [62, 63]. As consequence, bacteria overgrowth and the release of endotoxins, hydrogen sulfide, phenols, ammonia and indoles, expose intestinal mucosa and the host to harmful effects [63, 64]. The FODMAPs mechanism of action is linked to the stimulation of mechanoreceptors as a response to luminal distension from a combination of increased luminal water content from the osmotic effect, especially in the small intestine, and from the release of hydrogen and ammonia from the bacterial fermentation of saccharides. Such stimulation can lead to ascending messages that might be interpreted as abdominal pain or bloating; reflex responses to the diaphragm and anterior abdominal wall, leading to increased abdominal distension; and effects on motility with potential change in bowel habits. Furthermore, there could occur an excessive production of short-chain fatty acids, which could lead to visceral sensitivity and high-amplitude propagated colonic contractions, thus accelerating intestinal transit [64]. In this context, limiting the intake of the most

fermentable carbohydrates may have potentially alleviated FM symptoms, by reducing gases formation.

The first month of low FODMAPs diet seems to have been crucial to reduce SIBO and to optimize intestinal microbiota, allowing a greater efficacy of the posterior anti-inflammatory approach, and possibly of the pharmacological therapy that patient was already being subjected. The possible reduction of low-grade inflammation may be the explanation for the symptom's improvement experienced by intervention group.

Although it was also observed an improvement in gastrointestinal symptoms, fatigue and quality of sleep, in control group, the magnitude of the improvement was lower when compared to intervention group. These improvements in control group may be explained by the positive impact of WHO recommendations. However, once FM is associated with low-grade inflammation, those dietary recommendations *per se* do not seem to be anti-inflammatory enough.

This study has some limitations. The lack of a blood test for a low-grade inflammation specific cytokine such as IL-8, which has been associated with FM by several authors [2, 3], makes impossible to objectively determine the symptoms improvement mechanisms or to confirm the reduction in low-grade inflammation. Additionally, the absence of assessment at the end of the first month of intervention makes it impossible to objectively assess the impact of low FODMAPs diet alone, as well as the real need to carry it out in this context. It would be equally important to replicate this study, in order to amplify the sample.

Despite the proposed dietary restrictions, the diet was well accepted and was followed without difficulties. Additionally, the exclusion of gluten, sugar, ultra-processed products and dairy products in control group was confirmed at the end of the intervention. Taking into account the previous premises, we can say that the compliance of the participants is confirmed. Therefore,

the application of this nutritional strategy in clinical practice seems to be practicable and could be an important supporting tool for medical therapy in FM.

The present study allows us to conclude that an anti-inflammatory and low FODMAPs diet improved clinical features in this sample of FM patients, which may represent a relevant complement to the pharmacological therapy. The application of this nutritional strategy in clinical practice, with the possibility of further personalization, should be encouraged.

LIST OF ABBREVIATIONS

BPI – Brief Pain Inventory

FM - Fibromyalgia

FIQR – Fibromyalgia Impact Questionnaire Revised

FSS – Fatigue Severity Survey

PRO – Patient Reported Outcomes

PSQI – Pittsburg Sleep Quality Inventory

SF36 – Short-form 36

SF36_Mental – Short-form 36 for Mental Aspect

SF36_Physical - Short-form 36 for Physical Aspect

VAS – Visual Analogic Pain Scale

VAS_GI – Visual Analogic Scale for Gastrointestinal Symptoms

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Author contributor statement

ARS, AB, MFM, JVP, PM, MLS and PP conceived and planned the study design. ARS generated the random allocation sequence, enrolled participants, assigned participants to interventions and conducted the intervention. JVP, MLS and PP supervise the project. ARS, AB, MLS and PP performed statistical analysis. ARS wrote the first version of the manuscript and all authors revised it critically for important intellectual content. All authors provided

critical feedback, helped shape the research and analysis, and have read and approved the final manuscript.

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Conflict of interest statement

All the authors declare that there are no conflicts of interest.

Data sharing plan

Data described in the manuscript, code book, and analytic code will be made available upon request.

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Table 1. Baseline socio-demographic and lifestyle characteristics of the participants

Characteristics	Control Group (*n*= 24) Mean (±SD) Median (P25;P75)	Intervention Group (*n*= 22) Mean (±SD) Median (P25;P75)	*p*-value
Age (years)	56 (±8) 57 (51; 59)	60 (±6) 60 (56; 66)	0.057[a]
Disease duration (years)	13 (±9) 13 (4; 20)	14 (±8) 17 (5; 20)	0.526[a]
Body mass and composition			
Body mass index (kg/m^2)	30 (±6) 29 (26; 34)	29 (±4) 29 (25; 31)	0.531[a]
Waist circumference (cm)	99 (±14) 101 (90; 109)	98 (±10) 101 (89; 106)	0.783[a]
Fat mass (%)	39 (±9) 41 (33; 44)	39 (±6) 38 (34; 44)	0.796[a]
Muscle mass (kg)	24 (±3) 24 (21; 27)	23 (±2) 23 (21; 25)	0.502[b]
Total body water (%)	45 (±7) 43 (41; 50)	45 (±6) 45 (41; 47)	0.758[b]
	***n* (%)**	***n* (%)**	
Education (schooling)			
< 9 years	14 (60.9)	10 (45.5)	0.388
≥9 years	9 (39.1)	12 (54.5)	0.152
Work status			
Employed	10 (43.5)	8 (36.4)	0.541
Unemployed	3 (13.0)	1 (4.5)	0.344
Retired	5 (21.7)	8 (36.4)	0.248
Domestic / pensioner	5 (21.7)	5 (22.7)	0.327
Smoking habits			
Smoker	2 (8.7)	3 (13.6)	0.568
Nonsmoker	21 (91.3)	19 (86.4)	0.777
Alcoholic beverages consumption			
Daily	2 (8.7)	0 (0)	0.338
Occasional/Never	21 (91.3)	22 (100)	0.090
Exercise frequency			
<1 hour/week	22 (91.7)	18 (81.8)	0.596
≥1 hour/week	2 (8.3)	4 (18.2)	0.823

SD, standard deviation; P25, percentile 25; P75, percentile 75.

[a]*p*-value calculated by Independent-Samples T-Test between control and intervention groups mean values;

[b]*p*-value calculated by Mann-Whitney Test between control and intervention groups mean values.

Table 2. Clinical features in control and intervention group at baseline and post-intervention.

Outcomes	Control Group (n = 24)				Intervention Group (n = 22)			
	Baseline Mean (± SD) Median (P25;P75)	**Post-intervention Mean (± SD) Median (P25;P75)**	**Post-intervention - Baseline Difference Δ Mean (± SD) Δ Median (P25; P75)**	**Within-group analysis p-value**	**Baseline Mean (± SD) Median (P25;P75)**	**Post-intervention Mean (± SD) Median (P25;P75)**	**Post-intervention - Baseline Difference Δ Mean (± SD) Δ Median (P25; P75)**	**Within-group analysis p-value**
FIQR (Range: 0-100)	60.2 (±10.5) 60.5 (52.5; 68.9)	57.6 (±15.6) 61.2 (50.4; 68.4)	-2.2 (±16.1) -0.05 (9.1; 7.6)	0.515[b]	59.3(±9.2) 58.3 (53.3; 67.1)	39.5 (±21.8) 40.1 (23.8; 58.8)	-19.9 (±18.8) -15.8 (-34.2; -3.1)	p<0.001[b]
VAS (Range: 0-10)	7.6 (± 1.6) 8.0 (7.0; 8.8)	7.6 (±1.9) 8.0 (7.0; 9.0)	-0.04 (±2.1) 0.0 (-1.0; 1.0)	0.935[a]	7.7 (± 1.4) 8.0 (7.0; 9.0)	5.4 (±2.3) 6.0 (3.8; 7.3)	-2.3 (±2.5) -2.5 (-4.3; -0.8)	0.001[a]
VAS GI (Range: 0-10)	3.1 (± 1.4) 3.0 (1.9; 4.7)	2.3 (± 1.3) 2.2 (1.5; 2.6)	-0.9 (±1.3) -0.5 (-1.7; 1.7)	0.007[a]	3.4 (± 1.5) 3.4 (2.2; 4.4)	1.4 (±1.3) 1.2 (0.1; 2.6)	-2.0 (±0.9) -2.1 (-2.7; -1.3)	p<0.001[a]
BPI (Range: 0-20)	14.1 (± 2.2) 14.4 (12.9; 15.2)	13.0 (±3.6) 13.4 (11.1; 15.5)	-1.1 (±2.7) -1.0 (-2.4; 1.1)	0.062[b]	12.5 (± 2.3) 12.8 (10.8; 14.1)	8.7 (±4.7) 10.2 (4.4; 12.2)	-3.8 (±4.1) -3.2 (-5.7; -0.7)	p<0.001[b]
PSQI (Range: 0-21)	15.1 (± 4.0) 16.0 (12.0; 18.0)	13.9 (±4.5) 14.5 (11.0; 17.0)	-1.2 (±2.6) -1.0 (-2.8; 0.8)	0.037[b]	15.0 (± 5.2) 15.0 (10.8; 19.5)	11.6 (±5.7) 9.5 (8.5; 16.3)	-3.5 (±4.6) -3.0 (-8.0; 0.8)	0.002[b]
FSS (Range: 0-7)	6.4 (± 0.7) 7.0 (6.0; 7.0)	5.9 (±1.2) 6.0 (5.0; 7.0)	-0.5 (±1.0) 0.0 (-1.0; 0.0)	0.038[a]	5.5 (± 1.1) 6.0 (4.8; 6.0)	4.4 (±1.7) 5.0 (3.8; 5.3)	-1.1 (±1.2) -1.0 (-2.0; 0.0)	0.001[a]
SF36 (Range: 0-100)	38.6 (± 7.2) 38.9 (33.1; 42.7)	42.2 (±9.7) 42.2 (36.5; 47.2)	3.6 (±10.4) 2.2 (-4.9; 11.9)	0.137[a]	44.0 (±10.3) 42.6 (36.9; 53.5)	54.3 (±12.3) 58.4 (43.5; 63.6)	10.2 (±11.2) 9.0 (3.4; 15.9)	p<0.001[a]
SF36 Physical Component (Range: 0-100)	30.9 (±8.2) 31.8 (22.6; 35.6)	34.8 (±14.3) 30.9 (21.7; 49.6)	3.9 (±13.5) 1.3 (-4.8; 13.6)	0.168[b]	33.4 (±11.4) 34.6 (25.0; 41.0)	51.5 (±18.8) 56.3 (36.5; 66.7)	18.1 (±20.0) 22.5 (-1.0; 36.0)	p<0.001[b]
SF36 Mental Component (Range: 0-100)	38.6 (±15.8) 36.2 (26.2; 48.7)	47.2 (±19.8) 43.3 (28.9; 64.1)	8.5 (±23.1) 7.1 (-2.6; 24.7)	0.052[a]	54.4 (±23.1) 56.3 (33.7; 71.4)	63.4 (±21.4) 68.5 (51.3; 78.8)	8.9 (±21.0) 8.1 (1.9; 19.2)	0.023[a]

FIQR – Revised Fibromyalgia Impact Questionnaire; VAS – Visual Analogue Pain Scale; VAS GI - Visual Analogue Scale from gastrointestinal symptoms; BPI - Brief Pain Inventory; PSQI - Pittsburg Sleep Quality Index; FSS - Fatigue Severity Survey; SF36 - Short Form 36.

[a]p-value calculated by Wilcoxon Test between baseline and post-intervention, within-groups mean values;

[b]p-value calculated by Pared Sample T-Test between baseline and post-intervention, within-groups mean values.

Table 3. Between-group analysis of clinical features.

Outcomes	Between-group analysis		Between-group post-intervention – baseline difference analysis
	Baseline *p*-value	Post-intervention *p*-value	*p*-value
FIQR (Range: 0-100)	0.676[a]	0.004[a]	0.001[b]
VAS (Range: 0-10)	0.937 [a]	0.001[a]	0.002[b]
VAS GI (Range: 0-10)	0.660 [a]	0.023 [a]	0.002[b]
BPI (Range: 0-20)	0.015 [a]	0.001[a]	0.011[b]
PSQI (Range: 0-21)	0.808 [a]	0.073[a]	0.048[b]
FSS (Range: 0-7)	0.003 [a]	0.001[a]	0.042[a]
SF36 (Range: 0-100)	0.047 [a]	0.001[a]	0.045[a]
SF36 Physical Component (Range: 0-100)	0.454 [a]	0.002[a]	0.008[b]
SF36 Mental Component (Range: 0-100)	0.015 [a]	0.016[a]	0.947[b]

FIQR – Revised Fibromyalgia Impact Questionnaire; VAS – Visual Analogue Pain Scale; VAS GI - Visual Analogue Scale from gastrointestinal symptoms; BPI - Brief Pain Inventory; PSQI - Pittsburg Sleep Quality Index; FSS - Fatigue Severity Survey; SF36 - Short Form 36.

[a]*p*-value calculated by Mann-Whitney between control and intervention groups mean values;

[b]*p*-value calculated by T-Test for independent samples, between control and intervention mean values.

Table 4. Biochemical parameters assessment in control and intervention group at baseline and post-intervention.

Outcomes	Control Group (*n* = 24)				Intervention Group (*n* = 22)			
	Baseline Mean (± SD) Median (P25;P75)	**Post-intervention Mean (± SD) Median (P25;P75)**	**Post-intervention Δ Mean (± SD) Δ Median (P25; P75)**	**Within-group analysis *p*-value**	**Baseline Mean (± SD) Median (P25;P75)**	**Post-intervention Mean (± SD) Median (P25;P75)**	**Post-intervention Δ Mean (± SD) Δ Median (P25; P75)**	**Within-group analysis *p*-value**
hs-CRP (mg/dL)	0.33 (± 0.32) 0.24 (0.09; 0.43)	0.36 (± 0.44) 0.23 (0.09; 0.49)	0.03 (± 0.29) -0.03 (-0.15; 0.09)	0.920[a]	0.32 (± 0.27) 0.21 (0.11; 0.53)	0.37 (± 0.34) 0.19 (0.11; 0.62)	0.04 (± 0.26) -0.0 (0.08; 0.15)	0.745[a]
ESR (mm)	10.42 (±8.20) 7.5 (5.0; 14.5)	9.88 (± 8.83) 7.0 (5.0; 15.75)	-0.54 (± 4.90) - 0.5 (-3.0; 2.75)	0.663[a]	11.36 (± 8.29) 8.0 (5.0; 14.25)	11.64 (± 11.16) 8.50 (4.0; 13.75)	0.27 (± 6.69) 0.0 (-4.3; 3.25)	0.794[a]

hs-CRP – high-sensitive C-Reactive Protein; ESR – Erythrocyte Sedimentation Rate.
[a]*p*-value calculated by Wilcoxon Test between baseline and post-intervention, within-groups mean values;
[b]*p*-value calculated by Pared Sample T-Test between baseline and post-intervention, within-groups mean values.

Table 5. Between-group analysis of biochemical parameters.

Outcomes	Between-group analysis		Between-group post-intervention variation analysis *p*-value
	Baseline *p*-value	Post-intervention *p*-value	
hs-CRP (mg/dL)	0.886[a]	0.750[a]	0.567 [a]
ESR (mm)	0.650[a]	0.708[a]	0.640 [a]

hs-CRP – high-sensitive C-Reactive Protein; ESR – Erythrocyte Sedimentation Rate.
[a]*p*-value calculated by Mann-Whitney between control and intervention groups mean values;
[b]*p*-value calculated by T-Test for independent samples, between control and intervention mean values.

Table 1. Dietary intake in control and intervention group at baseline[1] and post-intervention[2].

Outcomes	Control Group (*n* = 24)				Intervention Group (*n* = 22)				Between-group analysis	
	Baseline Mean (± SD) Median (P25;P75)	**Post-intervention Mean (± SD) Median (P25;P75)**	**Post-intervention – Baseline Difference Δ Mean (± SD) Δ Median (P25; P75)**	**Within-group analysis *p*-value**	**Baseline Mean (± SD) Median (P25;P75)**	**Post-intervention Mean (± SD) Median (P25;P75)**	**Post-intervention – Baseline Difference Δ Mean (± SD) Δ Median (P25; P75)**	**Within-group analysis *p*-value**	**Baseline p-value**	**Post-intervention *p*-value**
Total energy intake (kcal)	1773 (±374) 1710 (1488; 2030)	1725 (±374) 1722 (1397; 1976)	-48.3 (±446.5) 68.7 (420.1; 229.4)	0.775[b]	1471 (±362) 1455 (1255; 1736)	1256 (±355) 1320 (1176; 1403)	-195.8 (±544) -13.9 (-412.3; 140.5)	0.236[b]	0.008[d]	$p<0.001$[d]
Protein (% TEI)	20 (±5) 19 (17; 23)	19 (±3) 20 (17; 22)	-1.2 (±4.9) -0.8 (-4.0; 2.2)	0.246[a]	21 (±4) 21 (19; 24)	19 (±3) 18 (17; 22)	-2.1 (±4.2) -1.6 (-4.8; 0.4)	0.030[a]	0.657[d]	0.777[c]
Carbohydrate (% TEI)	49 (±8) 50 (45; 55)	49 (±5) 50 (46; 52)	0.4 (±6.4) -0.2 (-4.2;3.9)	0.767[a]	51 (±9) 53 (44; 57)	46 (±6) 46 (41; 49)	-5.9 (±9.9) -4.9 (13.2; 1.4)	0.011[a]	0.385[d]	0.049[c]
Sugars (% TEI)	19.7 (±7.1) 20.0 (13.5; 23.8)	14.0 (±7.7) 15.2 (8.1; 19.5)	-5.7 (±8.9) -4.8 (-8.7; -0.7)	0.005[a]	19.8 (±7.6) 18.3 (14.2; 26.4)	12.3 (±7.1) 14.2 (9.2; 15.6)	-7.5 (±9.1) -7.6 (-14.6; -1.3)	0.001[a]	0.981[d]	0.416[c]
Monossacharides (% TEI)	5.0 (±2.3) 4.7 (2.9; 7.2)	5.2 (±2.9) 4.9 (3.2; 6.9)	0.2 (±3.3) 0.4 (-2.4; 2.9)	0.821[a]	4.9 (±2.9) 4.6 (2.6; 6.3)	5.5 (±3.4) 5.8 (3.6; 7.6)	-0.5 (±4.1) 0.9 (-2.4; 2.7)	0.542[a]	0.885[d]	0.778[c]
Dissacharides (% TEI)	4.6 (±3.0) 4.2 (2.1; 7.0)	4.2 (±2.5) 3.9 (2.6; 5.6)	0.4 (±2.6) 0.2 (-1.6; 1.8)	0.440[a]	4.9 (±2.6) 5.2 (2.6; 6.6)	1.7 (±1.3) 1.5 (0.9; 2.7)	-3.3 (±3.0) -3.5 (5.1; 0.9)	$p<0.001$[a]	0.737[d]	0.001[c]
Added sugars (% TEI)	0.8 (±1.6) 0.0 (0.0; 1.7)	0.7 (±0.9) 0.0 (0.0; 1.4)	-0.1 (±1.3) 0.0 (0.0; 0.3)	0.386[b]	0.5 (±1.4) 0.0 (0.0; 0.0)	0.0 (±0.0) 0.0 (0.0; 0.0)	-0.5 (±1.5) 0.0 (0.0; 0.0)	0.144[b]	0.186[c]	0.003[d]
Dietary fiber (g)	17.9 (±3.7) 17.7 (14.9; 20.3)	17.0 (±7.6) 18.3 (11.5; 21.7)	-0.9 (±8.1) 0.9 (-6.6; 3.6)	0.710[b]	16.0 (±5.6) 16.4 (10.7; 19.9)	16.5 (±9.5) 17.5 (13.6; 21.6)	0.5 (±11.5) 0.9 (-6.8; 7.3)	0.858[b]	0.235[c]	0.930[d]
Dietary fiber (g/1000 kcal)	1.7 (±0.4) 1.8 (1.5; 2.0)	1.9 (±0.5) 1.8 (1.3; 2.2)	0.1 (±0.6) 0.2 (-0.4; 0.4)	0.680[a]	1.6 (±0.6) 1.6 (1.0; 1.9)	2.0 (±0.6) 1.8 (1.6; 2.5)	0.4 (±0.9) 0.4 (-0.3; 0.8)	0.037[a]	0.169[d]	0.343[c]

[1]Values refer to 24h prior first contact (at baseline).

[2]Values are the average of the 3 days prior to the date of post intervention.

[3]Amount of food containing gluten in its composition (bread, biscuits, cake, pasta, savoury, breakfast cereals, cereal bars)

SFA = Saturated Fatty Acid; MUFA = Monounsaturated fatty acid; PUFA = Polyunsaturated fatty acid; n-3 = Omega 3 Fatty Acid; n-6 = Omega 6 Fatty Acid; TEI = Total Energy Intake.

[a]*p*-value calculated by Paired Samples T-Test between baseline and post-intervention, within-groups mean values;

[b] *p*-value calculated by Wilcoxon Test between baseline and post-intervention, within-groups mean values;
[c] *p*-value calculated by Independent-Samples T-Test between control and intervention groups mean values;
[d] *p*-value calculated by Mann-Whitney Test between control and intervention groups mean values.

Table 1. Dietary intake in control and intervention group at baseline[1] and post-intervention[2] (Cont.)

Outcomes	Control Group (*n* = 24)				Intervention Group (*n* = 22)				Between-group analysis	
	Baseline Mean (± SD) Median (P25;P75)	**Post-intervention Mean (± SD) Median (P25;P75)**	**Post-intervention – Baseline Difference Δ Mean (± SD) Δ Median (P25; P75)**	**Within-group analysis *p*-value**	**Baseline Mean (± SD) Median (P25;P75)**	**Post-intervention Mean (± SD) Median (P25;P75)**	**Post-intervention – Baseline Difference Δ Mean (± SD) Δ Median (P25; P75)**	**Within-group analysis *p*-value**	**Baseline *p*-value**	**Post-intervention *p*-value**
Total fat (% TEI)	30 (±6) 30 (26; 34)	31 (±6) 31 (27; 35)	1.2 (±7.4) -0.1 (-3.4; 7.9)	0.407[b]	28 (±8) 27 (23; 29)	37 (±7) 37 (32; 41)	9.4 (±9.8) 10.5 (0.6; 14.5)	0.001[b]	0.071[c]	0.004[c]
SFA (% TEI)	8.2 (±2.1) 8.3 (6.4; 10.4)	7.3 (±3.0) 7.5 (6.0; 10.1)	-0.9 (±2.9) 0.1 (-2.3; 0.9)	0.440[b]	7.8 (±2.5) 7.6 (6.2; 10.1)	4.9 (±2.7) 5.7 (3.6; 6.9)	-3.0 (±4.1) -2.3 (-6.6; 0.2)	0.006[b]	0.716[d]	0.004[d]
MUFA (% TEI)	5.7 (±2.5) 5.5 (4.2; 6.6)	4.8 (±2.1) 5.1 (3.9; 5.8)	-0.9 (±3.9) -0.3 (-2.1; 0.9)	0.331[b]	4.6 (±1.8) 4.6 (3.3; 5.4)	6.6 (±4.9) 5.9 (3.3; 9.0)	1.9 (±5.6) -0.8 (-1.7; 6.1)	0.123[b]	0.062[c]	0.117[c]
PUFA (% TEI)	13.0 (±4.5) 12.9 (10.2; 15.0)	13.9 (±5.2) 13.8 (11.9; 17.5)	0.8 (±7.3) 1.6 (-2.2; 5.9)	0.278[b]	11.2 (±5.2) 11.1 (7.3; 13.7)	16.2 (±8.4) 19.3 (14.4; 21.1)	5.0 (±10.1) 7.9 (1.2; 13.2)	0.022[b]	0.206[d]	0.018[d]
n-3 (% TEI)	0.9 (±0.6) 0.8 (0.5; 1.0)	0.9 (±0.5) 0.7 (0.6; 1.2)	-0.1 (±0.9) 0.1 (-0.2; 0.3)	0.530[b]	0.6 (±0.4) 0.4 (0.3; 0.6)	1.3 (±0.9) 1.3 (0.4; 1.9)	0.7 (±1.4) 0.4 (-0.4; 1.6)	0.046[b]	0.006[c]	0.538[d]
n- 6 (% TEI)	4.5 (±1.8) 4.2 (3.5; 5.1)	3.9 (±1.8) 4.1 (3.0; 4.8)	-0.7 (±2.9) -0.3 (1.6; 0.9)	0.317[b]	3.7 (±1.7) 3.6 (2.6; 4.2)	5.1 (3.9) 4.6 (2.5; 8.2)	1.4 (±4.5) 0.9 (-1.8; 5.6)	0.149[b]	0.129[d]	0.391[d]
Food containing gluten[3] (g)	179.8 (±92.4) 187.5 (105; 260)	150.9 (±54.9) 153.3 (116.3; 185)	-28.9 (±86.9) -14.2 (66.7; 28.3)	0.118[a]	170.6 (±71.8) 162.5 (118.8; 205)	0 0	-170.6 (±71.7) -162.5 (-205.0; -118.8)	$p<0.001$[b]	0.707[c]	$p<0.001$[d]
Dairy products (g)	303.1 (±210) 234.6 (131.3; 501.3)	254.3 (±216.3) 235 (55.4; 358.7)	-48.8 (±150.9) 150.9 (-144.8; 54.2)	0.127[a]	290.2 (±220.3) 220.0 (138.3; 411.3)	0 0	-290.2 (±220.3) -220 (-411.3; -138.8)	$p<0.001$[b]	0.848[c]	$p<0.001$[d]
Ultra-processed foods (g)	82.4 (±67.5) 67.2 (26.3; 142.5)	52.5 (±47.3) 47 (5.0; 78.8)	-29.9 (±78.6) -5.2 (-99.8; 17.5)	0.075[a]	47.3 (±44.1) 47.5 (0.0; 7.5)	0 0	-47.3 (±44.1) -47.5 (-75.0; 0.0)	$p<0.001$[b]	0.044[c]	$p<0.001$[d]
Sugar added to foods (g)	4.0 (±0.0) 6.34 (0.0; 8.0)	3.0 (±4.5) 0 (0; 8)	-1.0 (±4.3) 0 (0; 0)	0.257[b]	1.1 (±3.7) 0 (0; 0)	0 0	-1.1 (±3.7) 0 (0; 0)	$p<0.001$[b]	0.038[d]	$p<0.001$[d]

[1]Values refer to 24h prior first contact (at baseline).

[2]Values are the average of the 3 days prior to the date of post intervention.

[3]Amount of food containing gluten in its composition (bread, biscuits, cake, pasta, savoury, breakfast cereals, cereal bars)

SFA = Saturated Fatty Acid; MUFA = Monounsaturated fatty acid; PUFA = Polyunsaturated fatty acid; n-3 = Omega 3 Fatty Acid; n-6 = Omega 6 Fatty Acid; TEI = Total Energy Intake.

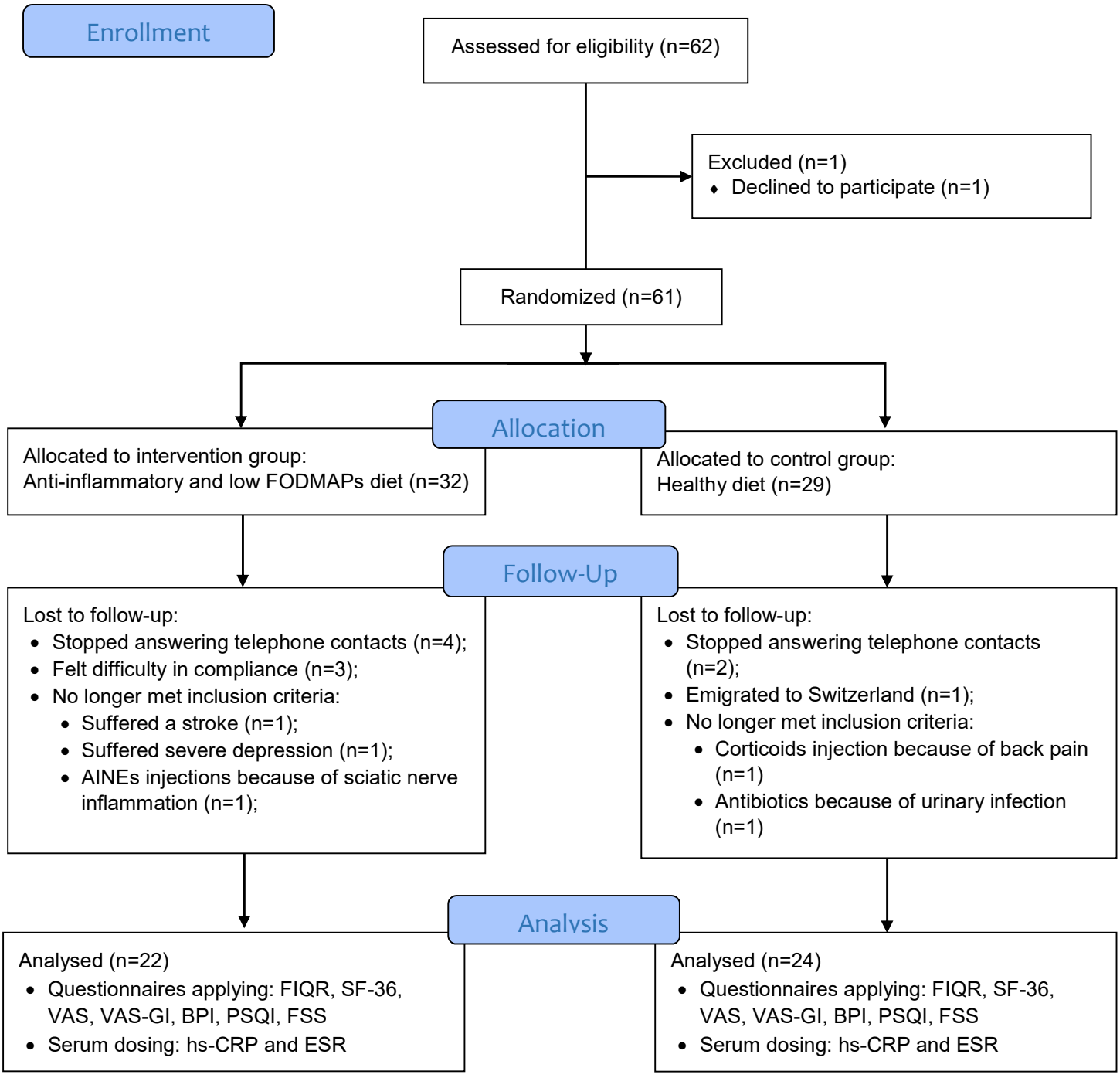
[a]*p*-value calculated by Paired Samples T-Test between baseline and post-intervention, within-groups mean values;

[b]*p*-value calculated by Wilcoxon Test between baseline and post-intervention, within-groups mean values;

[c]*p*-value calculated by Independent-Samples T-Test between control and intervention groups mean values;

[d]*p*-value calculated by Mann-Whitney Test between control and intervention groups mean values.

Supplementary Figure S1. CONSORT diagram of the study.



7. Suggestions for further research and clinical pratice

7. Suggestions for further research and clinical practice

It would be essential to continue research on this topic. For a disease with such a multiplicity of symptoms as FM, an approach that comprise all the possible action mechanisms involved will be essential. Our study brought very promising results. However, it would be important to expand the sample, in order to verify the effect of the intervention with greater statistical power.

Furthermore, it would be important to assess more objectively the impact of the intervention on low-grade inflammation. The lack of a blood test for a low-grade inflammation specific cytokine such as IL-8, which has been associated with FM by several authors [7, 63, 68] made it impossible to objectively determine the symptoms improvement mechanisms or to confirm the reduction in low-grade inflammation. Therefore, in the continuation of this investigation line, it would be of great importance to include this aspect.

It would also be relevant to evaluate the PRO at the end of the first month of intervention, to objectively assess the impact of low FODMAPs diet alone. In addition, it would be pertinent to assess the hydrogen exhalation to verify the presence of SIBO [136, 137], which would allow not only a more detailed study of this disease, but also a potentially personalization of the nutritional intervention in clinical practice.

In this context, it would be equally pertinent to investigate the microbiota composition, through 16S rRNA gene sequencing for species and strain-level microbiome analysis, to assess the possible differences between groups at the end of the study, and to examine the possible differences between baseline and post-intervention for intervention group. Additionally, through this analysis, it would be possible to investigate if there is a standard composition in FM patients.

The application of this nutritional strategy in clinical practice seems to be feasible and could be an important supporting tool for medical therapy in FM. From a clinical practice point of view, after applying the protocol, it would be interesting to assess food intolerance in respect to the excluded foods, thus allowing the creation of a more individualized nutritional approach.

Additionally, taking into account that all the symptoms of the disease are multifactorial in their possible origin, an integrative approach may make sense, where nutrition is added to the regular practice of physical exercise and stress management.

8. References

8. References

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