Gluten-Free Diet Reduces Pain in Women with Myofascial Pain in Masticatory Muscles: A Preliminary Randomized Controlled Trial

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Aims: To evaluate the efficacy of a gluten-free diet (GFD) as a treatment modality for pain management in women with chronic myofascial pain in masticatory muscles. **Methods:** In this randomized controlled trial, 39 female subjects were evaluated according to the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) and divided into three groups: a healthy group (n = 14; mean ± SD age = 34.57 ± 9.14 years); a control group (n = 12; age = 31.50 ± 7.38 years); and an experimental group (n = 13; age 30.00 ± 7.64 years). The outcome variables were: pain intensity, mechanical pain threshold (MPT), and pressure pain threshold (PPT). MPT was performed on the masseter muscle, and PPT was performed on both the masseter and anterior temporalis muscles. A nutritionist prescribed a 4-week individualized GFD for the experimental group. The healthy group was analyzed only initially, whereas the control and experimental groups were analyzed again after 4 weeks. Data were subjected to statistical analysis with a significance level of 5% (one-way analysis of variance followed by Bonferroni post hoc, paired *t*, Wilcoxon signed rank, Kruskal-Wallis/Dunn, and Pearson chi-square tests). **Results:** Participants who underwent a GFD showed reduction in pain intensity ($P = .006$) and an increase in PPT of the masseter ($P = .017$) and anterior temporalis ($P = .033$) muscles. The intervention did not influence the MPT of the masseter muscle ($P = .26$). In contrast, the control group showed no improvement in any parameter evaluated. **Conclusion:** GFD seemed to reduce pain sensitivity in women with TMD and may be beneficial as an adjunctive therapy for chronic myofascial pain in masticatory muscles; however, further studies in the fields of orofacial pain and nutrition are required.
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Dietary patterns have been identified as a cause of exacerbating painful chronic diseases,[1–4] such as temporomandibular disorders (TMD).[5] Individuals with TMD are known to have altered eating habits as a consequence of pain and/or dysfunction[6–8]; however, there are insufficient studies regarding the impact of diet on the maintenance, exacerbation, or reduction of orofacial pain.[9,10]

Gluten is the main structural protein of wheat and is formed by a complex mixture of proteins, mainly gliadin and glutenin.[11] Gliadin contains peptide sequences that are highly resistant to gastric, pancreatic, and intestinal proteolytic digestion[11] and has often been associated with cytotoxic effects, immunogenic effects, and an increase in intestinal permeability.[12,13]

Gluten intake has been highlighted as a cause of a variety of gastrointestinal, neurologic, dermatologic, psychologic, and musculoskeletal disorders.[14] Gluten ingestion can cause a cascade of immune responses, especially in celiac individuals.[13,15,16] Although these effects are more intense in celiac individuals,[13,15,16] scientific evidence has suggested that they occur even in nonceliac patients.[13,17,18]

After gliadin enters into the intestinal lumen through the ingestion of gluten-containing foods, this protein binds to the chemokine receptor CXCR3, promoting a series of events responsible for increased intestinal permeability and allowing paracellular translocation of gliadin.[15,16] After inflammatory processes have developed in the intestinal submucosa, in-

flammatory cytokines such as tumor necrosis factor (TNF)-α,[12,13] interleukin (IL)-12,[12] IL-15,[17] interferon (IFN)-γ, IL-6, and IL-8[13] can be found in patients' blood plasma, showing evidence of a systemic process.[13,18]

One study has suggested that a generalized inflammatory state may contribute to the pathophysiology of pain-related TMD.[19] Previous research evaluating chronic pain conditions such as fibromyalgia,[20] migraine,[21] and TMD[19,22,23] have found that these individuals have a plasma cytokine profile that differs from those of individuals without painful disorders.[19,23] Moreover, reports have suggested that the intake of gluten-containing foods may trigger an inflammatory response that starts in the intestine, reaches the bloodstream, and generates high concentrations of plasma cytokines.[13,18]

Chronic subinflammation has also been suggested as being the factor responsible for the increase in inflammatory markers and for the compensatory decrease or increase in anti-inflammatory markers in the plasma of patients with TMD.[19,22,23] Considering these findings, it may be assumed that those individuals could benefit from a gluten-free diet (GFD) because it may lead to the balance between these cytokines. To the present authors' knowledge, no previous study has evaluated the influence of gluten intake on the maintenance and/or exacerbation of orofacial pain or its elimination from diet as a treatment modality.

Somatosensory function is believed to provide important information regarding pain conditions and the prediction of therapeutic outcomes.[24] Subgroups of pain-related TMD show distinct somatosensory profiles when compared to those of healthy individuals,[24,25] and mechanical somatosensory assessment of the masticatory muscles is considered a reliable method to evaluate trigeminal sensory function.[26] Based on these findings, this pilot study aimed to evaluate the efficacy of GFD as a treatment modality for pain management in women with chronic myofascial pain in the masticatory muscles.

Materials and Methods

Trial Design and Ethics

This study was a preliminary parallel-arm randomized controlled trial developed at the Federal University of Ceará and approved by the local Human Research Committee under protocol number 2.439.297. All participants read and signed the Term of Free and Informed Consent before entering the study.

Participants

Due to sex-related differences in pain sensitivity[27] and a higher prevalence of TMD among women,[28] only female volunteers were included. Subjects in the age range between 18 and 55 years were recruited from among those seeking TMD treatment at the School of Dentistry, Federal University of Ceará, and also from among the general population, by means of social media, leaflets, and advertisements from March to December 2018, in the city of Fortaleza, Brazil. Exclusion criteria were volunteers presenting with toothache, fibromyalgia, frequent or chronic primary headache, history of facial trauma, systemic conditions such as diabetes mellitus and uncontrolled hypertension, systemic erythematosus lupus, Hansen disease, multiple sclerosis, hypothyroidism, carpal tunnel syndrome, intracranial hypertension, pregnancy, previously diagnosed disabling psychologic and neurologic disorders, history of chikungunya fever, and frequent use or abuse of licit or illicit drugs. Volunteers following a restrictive diet and/or with history of signs and symptoms of gluten intolerance, sensitivity, or wheat allergy,[14] or any other digestive pathology, were also excluded.

Subsequently, volunteers were evaluated according to the Research Diagnostic Criteria for TMD (RDC/TMD; the Portuguese version of the Diagnostic Criteria for TMD [DC/TMD] was not yet available when the study was conducted) for allocation into the three following groups: a positive control group, composed of asymptomatic women (healthy group); a negative control group, composed of women with TMD who underwent no intervention (control group); and an experimental group, composed of women with TMD who underwent a GFD (experimental group).

In addition to myofascial pain diagnosis according to the RDC/TMD, volunteers of the control and experimental groups had to have a moderate to severe pain complaint, graded as ≥ 5 on a numeric rating scale (NRS)[29] for a period of at least 3 months.[29] The major pain complaint had to be located in the masseter muscle, and volunteers had to have no history of treatment in the last 3 months. The healthy group consisted of age-matched volunteers who did not have any pain complaint.

Randomization and Blinding

A blinded statistician performed a random "yes" or "no" list in the Excel program in order to distribute TMD volunteers into the control and experimental groups (allocation ratio 1:1). Each volunteer received an identification number, and when they met the eligibility criteria, the randomization list was consulted. Those who received the term "no" were included in the control group, and those who received the term "yes" were included in the experimental group.

Three other researchers conducted the study: researcher #1 (S.M.A.N.) performed the evaluation of the volunteer's eligibility, TMD assessment according to the RDC/TMD, and group allocation according to

the randomization list; researcher #2 (J.A.O.B.; blinded) performed clinical evaluation (pain intensity and mechanical somatosensory assessment); and researcher #3 (M.P.S.) performed nutritional evaluation and GFD prescription.

Outcome Variables

Somatosensory evaluation. A standardized quantitative somatosensory evaluation was performed according to the German Research Network in Neuropathic Pain (DFNS),[30] and two mechanical parameters were evaluated: mechanical pain threshold (MPT), which was tested on the masseter muscle (body), and pressure pain threshold (PPT), which was tested on both the masseter muscle (body) and anterior temporalis muscle. There was a 5-minute interval between tests.

The healthy group underwent a single evaluation at baseline (T_1) by means of MPT and PPT sensory tests, whereas the control and experimental groups were evaluated twice, at T_1 and after 4 weeks (T_2). After the T_1 somatosensory evaluation, the control and experimental groups were asked to be present for the T_2 evaluation, and then to begin with a multimodal treatment (not part of the present study).

Participants could not use analgesic medication for at least 24 hours prior to somatosensory evaluations,[30,31] and they were not evaluated during their menstrual periods due to the possibility of sensitivity changes arising from hormonal alterations.[32] All procedures were conducted by researcher #2 (J.A.O.B.) in a quiet, temperature-controlled office.

Mechanical Pain Threshold

MPT was measured using Semmes-Weinstein monofilaments (North Coast Medical), which apply forces between 0.008 and 300 g/mm^2 to evaluate the function of Aδ fibers associated with a mechanical stimulus.[25] The monofilament was applied perpendicularly to the examination site, and the contact time was 1 to 2 seconds.[25] Participants were instructed to report the first perception of sharpness/pinprick. Using the method of limits technique, five suprathresholds and five subthresholds were established, with a series of stimuli with different ascending and descending intensities. The MPT was considered the geometric mean of these five series.[30]

Pressure Pain Threshold

PPT was recorded in order to evaluate deep pain sensitivity, mediated by the Aδ or C fibers.[25] This procedure was carried out by means of a digital algometer (DDK-20, Kratos Equipamentos Industriais), which at one end had a flat circular rod measuring 1 cm^2 in diameter, which was used to apply constant and increasing pressure at a rate of application of approximately 0.5 kgf/cm^2/second. Participants were instructed to press a button as soon as the first sensation of discomfort was perceived, and the value obtained was recorded.[31] PPT was determined as the mean value of two recordings in the same location,[31] with a 2-minute interval between them.

Pain Intensity

At T_1 and T_2, participants in both the control and experimental groups were asked to inform pain intensity according to a numeric rating scale (PI-NRS).[29]

Intervention

Nutritional protocol. Only the experimental group was submitted to a nutritional protocol, which was prescribed by researcher #3 (M.P.S.). A structured questionnaire was used to collect clinical and dietary data. Anthropometric measurements of weight using a stand-on anthropometric scale (Filizola) and height were assessed, and body mass index (BMI) was calculated using the formula BMI = weight (kg)/ height (m^2). Despite anthropometric assessment, it was emphasized that the aim of the study was to prescribe a GFD, not a calorie-restricted diet.[4]

To assess food intake, a 24-hour dietary recall was applied in which the participant was instructed to describe the diet during the previous day, as well as a habitual consumption recall describing the daily eating routine. From the results obtained, dietary changes were planned together with the volunteer, and, according to the patient's individual characteristics, foods that were a source of gluten were replaced by those lacking this protein in their nutritional composition, with due respect to sociocultural preferences and to establish equivalent portions in terms of calories and macronutrients.

In addition to the diet, each participant received a list of foods that could be eaten or should be excluded[4] and was instructed to complete a food diary for 7 days.[33] This record did not aim to analyze participants' intake of macro- and micronutrients, but rather to evaluate compliance with the GFD. Furthermore, participants were informed that judging their diets or promoting better eating habits were not the objectives of the study,[33] and that any changes other than those prescribed should be avoided.

Compliance with the GFD was also followed up weekly by telephone contact. Participants could also contact the nutritionist in case of any doubts. Moreover, prior to the T_2 somatosensory evaluation, the nutritionist analyzed each participant's food diary and performed another 24-hour dietary recall and habitual consumption recall relative to the last 4 weeks. Those who did not complete the food diary and/or consumed gluten-containing foods were excluded. In order to exclude any influence on eating behavior,[34] the control group did not undergo a nutritional evaluation.

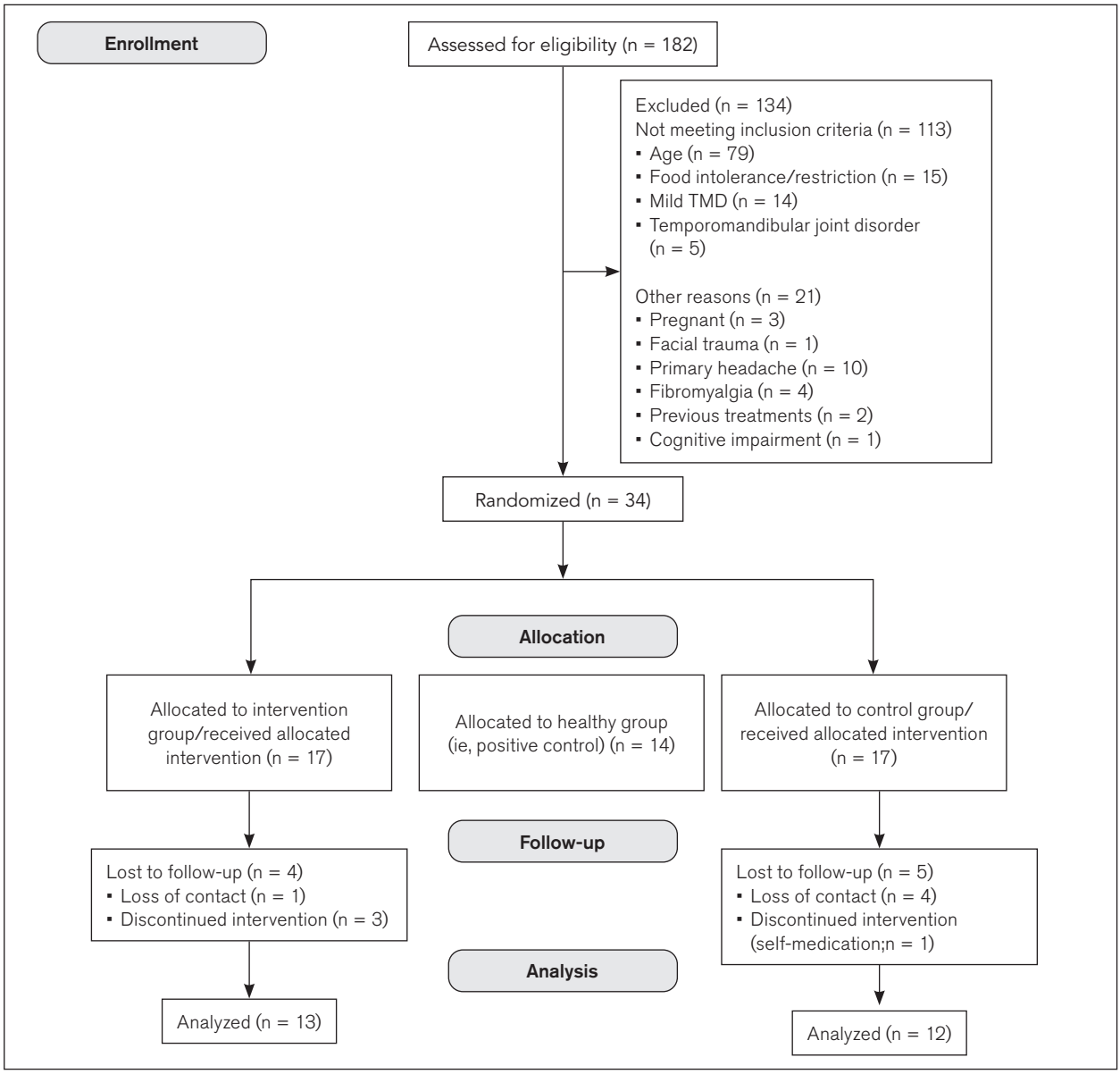

Fig 1 CONSORT flow of participant inclusion and study protocol.

Sample Calculation and Statistical Analysis

Based on pilot evaluations, it was found that a sample size of at least 11 subjects per group would be suitable to detect an NRS difference of 3.69, with SD of 3.24 (Wilcoxon test), in TMD volunteers treated with a 4-week GFD. A significance level of 5% and sample power of 90% were considered.

A per-protocol approach was applied for this trial. Somatosensory data were assessed for normal distribution using Kolmogorov-Smirnov test, and data were logarithmically (log 10) transformed before statistical analysis.[35]

One-way analysis of variance (ANOVA) followed by Bonferroni post hoc test were used to compare log-transformed MDT and PPT raw values of all study groups at T_1 and T_2. Paired *t* test was used to compare intragroup variations between T_1 and T_2. Wilcoxon signed-rank test was used to analyze PI-NRS variations between T_1 and T_2. Kruskal-Wallis/Dunn tests and Pearson chi-square test were used to analyze demographic characteristics. The significance level was set at 5% ($P = .05$), and statistical analysis were performed using SPSS software version 20.0 for Windows (IBM).

Results

Participants

A total of 39 participants completed the study (healthy group n = 14; control group n = 12; and experimental group n = 13). A flowchart detailing participant inclusion is presented in Fig 1. Sample characterization

Table 1 Demographics of Included Sample

	Healthy group	Control group	Experimental group	*P* value
Mean ± SD age, y	34.57 ± 9.14	31.50 ± 7.38	30.00 ± 7.64	.434[a]
Mean ± SD BMI, kg/m^2	24.69 ± 2.97	25.58 ± 4.73	24.83 ± 4.66	.819[a]
Race, n (%)				
White	6 (42.9)	4 (33.3)	4 (30.8)	.610[b]
Black	0	0	1 (7.7)	
Brown	8 (57.1)	8 (66.7)	7 (53.8)	
Asian	0	0	1 (7.7)	
Education, n (%)				
Primary school	0	0	1 (7.7)	.267[b]
High school	4 (28.6)	5 (41.7)	6 (46.2)	
Incomplete university education	1 (7.1)	2 (16.7)	3 (23.1)	
University education	6 (42.9)	5 (41.7)	3 (23.1)	
Postgraduate student	3 (21.4)	0	0	
Marital status, n (%)				
Single	8 (57.1)	6 (50.0)	6 (46.2)	.692[b]
Married	6 (42.9)	6 (50.0)	6 (46.2)	
Widow	0	0	1 (7.7)	
Job, n (%)				
No	5 (35.7)	4 (33.3)	1 (7.7)	.191[b]
Yes	9 (64.3)	8 (66.7)	12 (92.3)	

BMI = body mass index.
P < .05 was considered significant.
[a]Kruskal-Wallis/Dunn test.
[b]Pearson chi-square test.

Table 2 Summary of Wilcoxon Signed-Rank Test Results for Pain Intensity According to Numeric Rating Scale (PI-NRS) at T_1 and T_2

							Wilcoxon signed-rank test	
Group		No.	Mean rank	Sum of ranks	NRS T_1 Mean ± SD	NRS T_2 Mean ± SD	NRS T_2–T_1	*P* value
Control	Negative ranks	5[a]	5.00	25.00	6.63 ± 1.40	7.69 ± 1.49	–0.302[d]	.763
	Positive ranks	4[b]	5.00	20.00				
	Ties	3[c]						
	Total	12						
Experimental	Negative ranks	11[a]	7.68	84.50	6.33 ± 2.71	4.00 ± 2.61	–2.728[d]	.006*
	Positive ranks	2[b]	3.25	6.50				
	Ties	0[c]						
	Total	13						

[a] NRS T_2 < NRS T_1; [b] NRS T_2 > NRS T_1; [c] NRS T_2 = NRS T_1.
[d] Based on positive ranks.
*Significant difference (*P* < .05).

of each group is reported in Table 1, demonstrating similarity between groups at baseline.

Pain Intensity

At T_1, no significant differences in PI-NRS were observed between the control and experimental groups, suggesting an effective randomization. After the intervention, the experimental group showed an improvement in pain intensity (*P* = .006; Table 2).

Somatosensory Evaluation

Data of the somatosensory evaluation are shown in Table 3. The pain thresholds of the control and experimental groups were lower than those in the healthy group. In the intragroup analysis, the experimental group showed an increase in PPT of the masseter muscle (*P* = .017) and anterior temporalis muscle (*P* = .033). MPT of the masseter muscle was not influenced by withdrawal of gluten from the diet (*P* = .26).

Table 3 Mean ± SD Raw and Log-Transformed Values of Mechanical Somatosensory Testing

			T_1		*P*	T_2		*P*	*P* value[e] (T_1 x T_2)	
		Healthy	Control	Experimental	value[c]	Control	Experimental	value[d]	Control	Experimental
MPT: Masseter	Raw	35.11 ± 30.99	10.28 ± 16.84	12.69 ± 20.50		11.53 ± 15.78	20.92 ± 43.94			
	Log	1.27 ± 0.63	0.56 ± 0.73[a]	0.50 ± 0.80[a]	.015	0.67 ± 0.68	0.71 ± 0.78	.059	0.544	.26
PPT: Masseter	Raw	1.14 ± 0.31	0.67 ± 0.28	0.57 ± 0.37		0.69 ± 0.29	0.76 ± 0.35			
	Log	0.04 ± 0.12	−0.22 ± 0.20[a]	−0.36 ± 0.37[a]	.001	−0.22 ± 0.25[a]	−0.17 ± 0.22[a]	.005	0.997	.017[b]
PPT: Anterior temporalis	Raw	1.50 ± 0.39	0.93 ± 0.36	0.78 ± 0.47		0.85 ± 0.35	1.01 ± 0.47			
	Log	0.16 ± 0.11	−0.06 ± 0.17[a]	−0.21 ± 0.34[a]	.001	−0.10 ± 0.17[a]	−0.05 ± 0.22[a]	.001	0.539	.033[b]

MPT = mechanical pain threshold; PPT = pressure pain threshold.
[a]Significantly different vs healthy group at T_1. [b]Significantly different for T_1 vs T_2.
[c]One-way ANOVA/Bonferroni test (healthy group x control group x experimental group at T_1).
[d]One-way ANOVA/Bonferroni test (healthy group x control group x experimental group at T_2).
[e]*t* test (intragroup comparison for T_1 x T_2).

Discussion

To the best of the present authors' knowledge, this study was the first to evaluate the influence of a GFD in individuals with TMD. In order to do so, women with myofascial pain in masticatory muscles were allocated into two groups, one of which was submitted to a GFD for 4 weeks. When compared to healthy participants by means of MPT and PPT tests, the control and experimental groups showed increased pain sensitivity. After intervention with GFD, the experimental group exhibited a reduction in PI-NRS and an increase in PPT of the masseter and anterior temporalis muscles. At baseline, the groups were similar for age, BMI, ethnicity, education, marital, and occupational status, excluding any possible influence of demographic characteristics on the results.[28]

Nevertheless, the data presented here should be analyzed with caution. Participants who received a GFD intervention showed improvement in pain sensitivity, and although a reduction of approximately 2 points on the NRS is considered clinically important,[36] the experimental group continued to show lower PPT values when compared to healthy individuals. Because TMD has several underlying pain-related mechanisms,[37] it should not be expected that a GFD alone would cause complete remission of painful symptoms. Moreover, although the GFD itself may not have been the factor exclusively responsible for improvement in the experimental group, the control group did not show any fluctuation in pain symptoms after 4 weeks. Therefore, if the results presented here represent an effect of GFD, the mechanisms involved require elucidation.

Mechanical hyperalgesia is the somatosensory abnormality most frequently found in individuals with TMD.[25] Therefore, tests such as MPT and PPT are expected to show lower values than those exhibited by healthy individuals. The increase in PPT of the masseter and anterior temporalis muscles may suggest that gluten withdrawal from the diet reduced Aδ and C fiber sensitization. The GFD may perhaps have altered plasma cytokine levels, resulting in increased pain thresholds.

When compared to healthy subjects, individuals with TMD were shown to have increased plasma levels of IL-1β, IL-6, and TNF-α, especially those with a high level of disability, according to the Graded Chronic Pain Scale (RDC/TMD).[23] Association between plasma IL-8 and widespread pain tenderness has also been found in individuals with TMD,[19] suggesting a possible role of this cytokine in mechanical hyperalgesia. Furthermore, variation in the anti-inflammatory profile was also observed in TMD patients, in whom high concentrations of IL-10[23] and IL-1ra,[19] and reduction in Omentin-1, were found.[22]

The present study did not evaluate those parameters; however, studies that have evaluated gluten intake suggested alterations in plasma pro- and anti-inflammatory cytokine levels, such as an increase in plasma concentrations of anti-inflammatory cytokines IL-10 and IL-13,[13] as well as glycoprotein α-2-macroglobulin.[18] These cytokines can bind to various inflammatory cytokines, including IL-1β, IL-6, and TNF-α, causing their biologic activities to be totally or partially inhibited.[38] Although previous findings have suggested that gluten ingestion does not cause symptoms in individuals who do not have a physiologic susceptibility to it (ie, gluten-related disorders),[39] it has also been suggested that a 4-week GFD may alter the structure of gut microbiota and the associated immune function in healthy individuals.[40] This occurs by inducing reduced pro- and anti-inflammatory signaling in the gut through lowered production of pro-inflammatory cytokines TNF-α, IFN-γ, and chemokine IL-8, as well as lowered production of the anti-inflammatory cytokine IL-10.[40] For future studies, the evaluation of plasmatic cytokine levels within a 4-week GFD and a longer follow-up period are suggested.

The present study has limitations. The control group did not undergo a nutritional protocol, since

this could encourage the participants to change their diet. Evidence has suggested that during dietetic research periods, subjects often change their food patterns even after being told to maintain a normal dietary pattern.[34] In the present study, the additional attention received by the experimental group may have led to research bias. During the 4-week intervention, participants were asked to avoid changes in food intake patterns other than those prescribed, and any type of restrictive diet, including the restriction of gluten intake, was an exclusion criterion. However, the amount and frequency of gluten consumption and food texture were not assessed at baseline. Notwithstanding, a 7-day food diary record previous to the intervention[41] is suggested for future studies in order to evaluate participants' diet in terms of food texture, intake of gluten, energy, and nutrients at baseline. To estimate gluten consumption more precisely, participants' diet may also be examined by using a food frequency questionnaire.[18] Comparative analysis between these data and those collected when undergoing a GFD is also suggested to verify possible alterations in nutritional intake due to the intervention. Problems that may be found in dietary exclusion studies—such as collinearity (ie, compensatory changes in other components due to changing one component of the diet) and changes in unmeasured dietary substrates—that could contribute to the findings observed[41] may have occurred in this investigation.

In the present study, participants received detailed information about how to comply fully with the GFD, and those who reported gluten consumption were excluded; however, participants' total compliance with the diet cannot be ensured. Dietary interviews are often difficult to standardize, as they are subjective or rely on a truthful response from the patient and are unable to identify involuntary mistakes.[42] Future studies with more objective parameters, such as measurement of gluten in stool and urine samples,[43] may be useful to determine diet compliance.

The popularity of a GFD may also have affected the study. Social media, consumer-directed marketing by manufacturers, and scientific literature make some people believe that gluten avoidance may have immediate health benefits, such as weight loss and preventing the development of future diseases.[44] In the present study, those beliefs may have influenced the expectations of the experimental group regarding the intervention.

The risk of bias inherent with somatosensory assessment and its subjectivity may have influenced the results presented herein.[35] Furthermore, this pilot trial was developed in a Brazilian population, and influences of race and sociocultural factors must be considered; multicenter research is required to better assess these confounding variables. For all groups, PPT values were below those previously reported in recent literature.[26,31,45] Therefore, although factors such as sex and ethnicity are known to influence pain sensitivity,[46] other elements such as participants' cognition, instructions, examiner, and site of test application may have been sources of variability.[47] In the present study, a single trained rater performed somatosensory evaluation by using standardized instructions and an application of a stimulus. For future studies, the establishment of screening tools to exclude patients who are unlikely to be able to participate in somatosensory testing and establishment of normative data may be useful.[47]

Additionally, reports have also indicated that psychologic factors could influence somatosensory responses,[48] and future studies should also include psychologic evaluations, such as those suggested in IMMPACT recommendations for patient phenotyping[49] and core outcomes for clinical trials focused on chronic pain.[29,50] Other lifestyle factors, such as beginning with physical activity or altering its intensity, should also be assessed due to their influence on pain modulation.[51]

As was the case in the study of Aziz et al,[52] which evaluated the efficacy of a GFD in subjects with irritable bowel syndrome/diarrhea, this was a real-life pragmatic study where the responsibility was placed on the patients to undergo a GFD after a single nutritional evaluation, rather than patients being in an extremely controlled study environment, such as those where meals are provided. However, since the experimental group participants who consumed gluten-containing foods were excluded, future studies using an intention-to-treat analysis should be performed in order to promote a more realistic scenario.

According to limitations addressed in this preliminary study, double-blinded placebo-controlled trials are proposed for future studies as an alternative in order to reduce potential bias. For this study design, all subjects should adhere to a GFD and then be randomly allocated into two groups. While continuing the GFD, participants from one group should receive supplements containing gluten, while participants from the other group should receive gluten-free supplements. Double-blinded placebo-controlled challenges with crossover present a high level of evidence for assessing gluten-related disorders.[53] The gluten challenge involves two stages: *(1)* the assessment of clinical response to GFD; and *(2)* the assessment of effects associated with reintroducing gluten after a period of GFD.[53] However, determining a suitable protocol for gluten challenge in TMD patients is still premature, since several factors should be considered when it is undertaken, such as the amount of gluten used, the time of gluten exposure, and washout periods.[54]

Finally, this was a preliminary study, and TMD patients should not be encouraged to undertake self-prescription of gluten withdrawal. A GFD has been associated with reduction in the population of beneficial gut bacteria,[40] deficiencies of micronutrients and fiber, increases in fat content of foods, and even coronary artery disease.[44] Furthermore, it may lead to social isolation, negative psychosocial impacts,[44] and economic burden, since gluten-free products are significantly more expensive than their wheat-based counterparts.[55]

Conclusions

The present study suggested that GFD seemed to reduce pain in women with myofascial pain in masticatory muscles and may be beneficial as an adjunctive therapy for its management. However, due to its preliminary nature, future double-blinded placebo-controlled clinical trials are required to confirm these findings.

Clinical Research Highlights

- GFD reduced reported pain intensity in women with myofascial pain in masticatory muscles.
- GFD increased PPT of the masseter muscle in women with myofascial pain in masticatory muscles.
- GFD increased PPT of the anterior temporalis in women with myofascial pain in masticatory muscles.

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