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FUNCTIONAL DISORDERS

The Association Between a Mediterranean Diet and Symptoms of Irritable Bowel Syndrome

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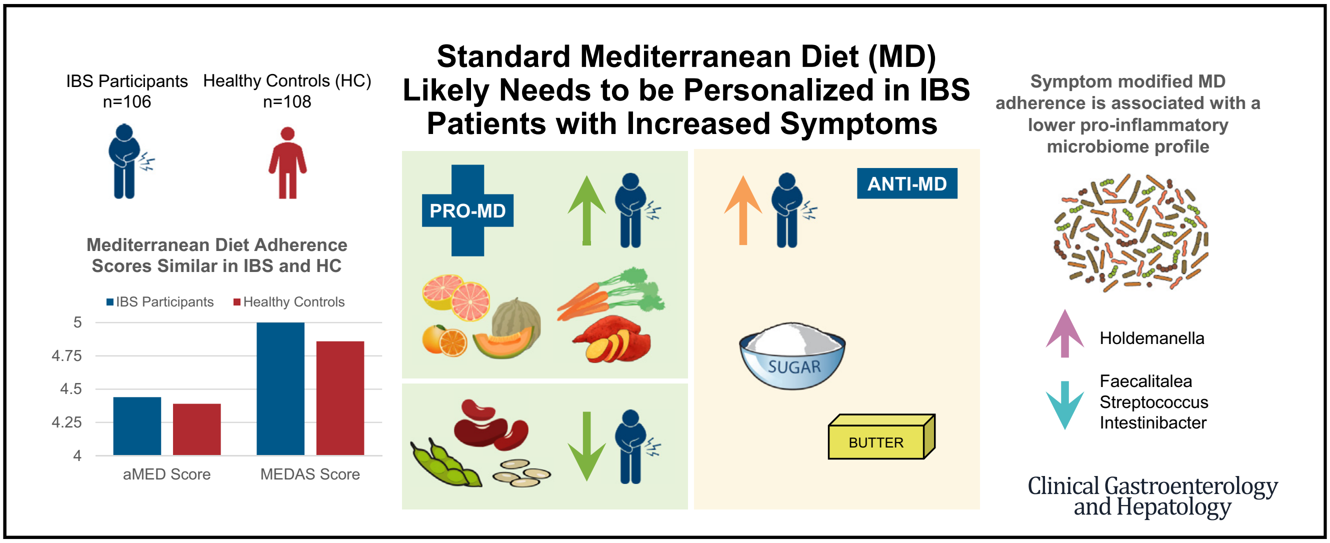

BACKGROUND & AIMS: Low adherence to Mediterranean diet (MD) has been shown to be associated with a higher prevalence of irritable bowel syndrome (IBS), but its association with IBS symptoms is not established. We aim to assess the association between MD and IBS symptoms, identify components of MD associated with IBS symptoms, and determine if a symptom-modified MD is associated with changes in the gut microbiome.

METHODS: One hundred and six Rome +IBS and 108 health control participants completed diet history and gastrointestinal symptom questionnaires. Adherence to MD was measured using Alternate Mediterranean Diet and Mediterranean Diet Adherence Screener. Sparse partial least squares analysis identified MD food items associated with IBS symptoms. Stool samples were collected for 16S ribosomal RNA gene sequencing and microbial composition analysis in IBS subjects.

RESULTS: Alternate Mediterranean Diet and Mediterranean Diet Adherence Screener scores were similar between IBS and health control subjects and did not correlate with Irritable Bowel Syndrome Severity Scoring System, abdominal pain, or bloating. Among IBS participants, a higher consumption of fruits, vegetables, sugar, and butter was associated with a greater severity of IBS symptoms. Multivariate analysis identified several MD foods to be associated with increased IBS

Abbreviations used in this paper: aMED, alternate Mediterranean diet; aMED-m, symptom modified-aMED; DHQ II, Diet History Questionnaire II; FODMAP, fermentable oligo-, di-, monosaccharides and polyols; GI, gastrointestinal; HC, healthy control subjects; IBS, irritable bowel syndrome; IBS-SSS, Irritable Bowel Syndrome Severity Scoring System; MD, Mediterranean diet; MEDAS, Mediterranean Diet Adherence Screener; VSI, Visceral Sensitivity Index.

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symptoms. A higher adherence to symptom-modified MD was associated with a lower abundance of potentially harmful *Faecalitalea*, *Streptococcus*, and *Intestinibacter*, and higher abundance of potentially beneficial *Holdemanella* from the Firmicutes phylum.

CONCLUSIONS: **A standard MD was not associated with IBS symptom severity, although certain MD foods were associated with increased IBS symptoms. Our study suggests that standard MD may not be suitable for all patients with IBS and likely needs to be personalized in those with increased symptoms.**

Keywords: Mediterranean Diet; Irritable Bowel Syndrome; Symptoms; Microbiome.

Mediterranean-style diet (MD) is the traditional eating habits of people in countries bordering the Mediterranean Sea. It is considered a healthy lifestyle characterized by high intake of whole grains, fruits, vegetables, nuts, and seeds. Fish and other seafood, poultry, and dairy are eaten in moderation, and red meat and foods high in sugar are eaten on occasion. Olive oil is the main fat source in an MD.[1]

Growing evidence indicates that the consumption of the MD can reduce the risk of cardiovascular disease, diabetes, and cancer through the antioxidant-rich and anti-inflammatory properties of its essential foods.[2,3] There are several properties of the MD that may promote gut health. High amounts of phenols in the MD diet have been shown to have anti-inflammatory properties including decreased expression of inflammatory molecules.[4] In addition, MD is associated with increased abundance of short-chain fatty acid–producing microbiota, which help to maintain proper function of the intestinal epithelium.[5] Furthermore, olive oil has been shown to be associated with decreased intestinal inflammation and visceral hypersensitivity in animal studies.[6,7]

Irritable bowel syndrome (IBS) is a common gastrointestinal (GI) condition affecting approximately 4%–11% of the global population.[8] The pathogenesis of IBS is multifactorial reflecting a combination of various host and environmental factors.[9] Not only does diet contribute to the pathogenesis of IBS, but most patients with IBS identify foods as a trigger to their IBS symptoms.[10] Dietary interventions, such as the low fermentable oligo-, di-, monosaccharides and polyols (FODMAP) diet are considered first-line treatments for IBS.[11] However, most dietary interventions focus on elimination of trigger foods and can be restrictive, difficult to maintain, may lead to deficiencies certain nutrients, and change intestinal microbiota negatively.[12,13] Therefore, more balanced dietary guidelines are needed for dietary management of IBS.

Although the MD is a well-balanced diet in contrast to more restrictive diets (eg, low FODMAP, gluten-free), few studies have examined the association between MD and IBS.[14-16] Limited data suggest that low adherence to an MD is associated with a higher prevalence of IBS and other disorders of gut-brain interaction, but the association between MD and IBS symptoms has not been well investigated. Furthermore, there are currently no studies on the contribution of individual MD foods to IBS symptoms to determine if certain components of the MD diet should be modified for patients with IBS.

The aims of our study are to (1) compare MD adherence in participants with IBS and healthy control subjects (HCs); (2) determine if MD correlates with severity of IBS symptoms, including abdominal pain, bloating, and overall IBS symptoms; (3) identify components of the MD that correlate with severity of IBS symptoms; and (4) determine if symptom-modified MD is associated with a difference in microbiome profile. We hypothesized that in general, adherence to the MD would not differ in IBS and HCs, but patients with IBS who had greater adherence to an MD would have decreased IBS symptom severity and a different fecal microbiome profile than patients with IBS who were less adherent to an MD. Additionally, we hypothesized that there may be some individual MD foods (eg, high FODMAP foods) that would be associated with greater IBS symptom severity.

Methods

Participants

This study was a cross-sectional study including retrospective analysis of adult participants with IBS and HCs who participated in IBS clinical research studies conducted between July 2013 and November 2021 at G. Oppenheimer Center for Neurobiology of Stress and Resilience at the University of California, Los Angeles.

The diagnosis of IBS was made using the Rome III or Rome IV[17,18] criteria depending on the time of recruitment. HCs had no history of GI symptoms or disease. Participants with organic GI diseases were excluded. Participants who submitted stool samples for microbiota analysis were also excluded if they received antibiotics within the previous 3 months. Additional details regarding inclusion and exclusion criteria were published previously; however, assessment of MD adherence and its relationship to IBS symptoms are novel and not previously published.[13]

This study was approved by the University of California, Los Angeles institutional review board.

Irritable Bowel Syndrome Symptom-Related Questionnaires

IBS participants completed validated GI symptom-related questionnaires including Bowel Symptom Questionnaire[19] and Irritable Bowel Syndrome Severity Scoring System (IBS-SSS)[20] (Supplementary Methods).

Psychological Symptoms Assessment

Participants completed the validated questionnaires: Visceral Sensitivity Index (VSI),[21] which measures GI symptom-related anxiety; and Hospital Anxiety and Depression Scale,[22] which measures current anxiety or depression symptoms (Supplementary Methods).

Dietary Assessment

Dietary information was obtained through the Diet History Questionnaire II (DHQ II).[23] MD adherence was assessed by validated Alternate Mediterranean Diet (aMED) and Mediterranean Diet Adherence Screener (MEDAS) scores. aMED was an adaptation of the traditional MD to non-MD countries,[24] whereas MEDAS was used to guide the PREDIMED trial, one of the leading studies of the MD.[3] Alternative Healthy Eating Index-2010 was also calculated for each participant to assess for diet quality because it was designed to reduce chronic disease risks (Supplementary Tables 1-3).[25]

In addition, a GI dietitian identified DHQ II food groups and individual food items that were either consumed regularly or avoided/consumed occasionally (<1 serving a day) in an MD for further analysis based on a combination of Dietary Inflammatory Index and the scoring guide used in the PREDIMED study (Supplementary Table 4).[3,26] We termed these foods "pro-MD foods" and "anti-MD foods," respectively. The average total daily consumption of these foods was calculated from the DHQ II data using the Diet*Calc software[27] and reported as grams per day.

Statistical Analysis

IBS versus HC group comparisons for demographic characteristics, MD adherence scores, and food items were performed using independent t-tests, general linear model, or chi-square tests. Generalized logistic regression (link logit) with IBS status as a dependent variable was used to determine the group differences adjusting for Hospital Anxiety and Depression Scale-Anxiety. General linear model was used to determine the association between IBS symptoms and MD adherence. Statistical significance was defined as $P < .05$. Sparse partial least squares regression implemented in mixOmics R package was applied to determine the relationships between dietary intake of anti-MD and pro-MD foods with IBS symptom severity measures (Supplementary Methods).

> **What You Need to Know**
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> **Background**
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> The Mediterranean diet is considered to be a healthy diet. Although few studies show that Mediterranean diet adherence is inversely associated with incidence of IBS, the association between Mediterranean diet and IBS symptoms is not well established.
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> **Findings**
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> Adherence to the Mediterranean diet was not associated with severity of IBS symptoms. In addition, food groups such as fruits and vegetables were associated with increased IBS symptoms. Symptom modified Mediterranean diet was associated with more beneficial gut microbiome profile.
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> **Implications for patient care**
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> An IBS-modified Mediterranean diet, rather than a standard Mediterranean diet, should be considered for the management of IBS symptoms.

Based on the dietary variables selected by the model and their correlation with IBS symptoms, a symptom modified-aMED (aMED-m) score was calculated by omitting the pro-MD food items associated with increased IBS symptoms from the standard aMED calculations. We compared differences in gut microbiome in those with low, medium, and high aMED-m scores.

Microbiome Analysis

DNA was extracted from fresh frozen stool samples using the PowerSoil DNA Isolation Kit (MO BIO, Carlsbad, CA). The V4 hypervariable region was amplified using the 515F and 806R primer set. DNA was then purified using a commercial kit and the DNA was sequenced using an Illumina HiSeq 2500 (Illumina, San Diego, CA). The raw reads were then processed through DADA2 using default parameters to generate amplicon sequence variants. Taxonomic assignment was performed using the Silva 138 database. Low abundant amplicon sequence variants were removed if they did not have a relative abundance of greater than 1E-7. The mean reads per sample was 57,462 with a standard deviation of 25,423. Alpha diversity and beta diversity was calculated using QIIME 2. The distance metric used for beta diversity was the robust Aitchison from the DEICODE plugin in QIIME 2. Significance for beta diversity was calculated using the Adonis package in R, which uses permutational multivariate analysis of variance. Beta diversity was visualized using principal coordinate analysis plots. Alpha diversity was measured using a measurement of species evenness (Shannon Index) and richness (Chao1 index). Differential abundance testing was performed using DESeq2 in R, which uses a negative binomial modeling to test nonrarefied count data. P values were converted to q-values to correct for multiple hypothesis testing.

Results

IBS participants and HCs were similar in age, sex, body mass index, and race (Table 1). However, IBS participants had higher Hospital Anxiety and Depression Scale-Anxiety scores compared with HCs (7.94 vs 4.23; $P = 3.0e\text{-}11$) and were more likely to consume a restrictive diet than HCs (14% vs 4%; $P = 5.90e\text{-}05$).

There were no significant differences in mean aMED and MEDAS scores between IBS participants and HCs (M [standard deviation (SD)] 4.44 [1.82] vs 4.39 [1.80], $P =$ .83; and 5.0 [1.37] vs 4.86 [1.40], $P = .46$). There were also no significant associations between adherence to MD and overall IBS symptoms, abdominal pain, bloating, VSI, and IBS-SSS (Table 2). In terms of diet quality, aMED and MEDAS scores were positively associated with Alternative Healthy Eating Index scores ($r = 0.54$, t[dof] = 9.40 [212], $P < 2.2e\text{-}16$; and $r = 0.45$, t[dof] = 7.26 [212], $P = 7.19e\text{-}12$, respectively). Overall, 21% of IBS participants and 9% HCs were of Mediterranean descent ($P = .18$). However, the aMED and MEDAS scores were not different between our participants of Mediterranean descent countries compared with those with any other country of origin (M [standard deviation (SD)] 4.36 [1.78] vs 4.35 [1.89], $P = .98$; and 4.87 [1.39] vs 5.13 [1.41], $P = .35$).

For pro-MD and anti-MD food groups, IBS participants, on average, consumed less beans compared with HCs ($P = .048$) (Table 3). Furthermore, in IBS participants, fruits were associated with higher abdominal pain, bloating, and IBS-SSS; and vegetables were associated with higher VSI scores (all $P < .05$). However, higher consumption of beans, legumes, and soy (pro-MD) was associated with lower overall symptoms and IBS-SSS ($P = .0004$ and .002, respectively) but not with VSI. Higher consumption of anti-MD food groups, such as added sugar, was associated with higher abdominal pain ratings; and higher consumption of butter, creams, and

Table 1. Demographic Characteristics

	IBS (N = 106)	HCS (N = 108)	*P* VALUE
Age	28.75 (10.93)	28.85 (10.43)	.95
Sex, n (%)	77 (73)	84 (78)	.43
BMI	23.88 (4.03)	24.73 (3.59)	.1
HADS-Anxiety	7.94 (4.24)	4.23 (3.41)	3.0e-11
Race, n (%)			.61
Asian	26 (24)	38 (35)	
Black	7 (6)	9 (8)	
White	56 (53)	42 (39)	
Multiracial	10 (9)	11 (10)	
Not available	7 (7)	8 (7)	
Hispanic, n (%)	26 (24)	22 (20)	.34
aMED score	4.44 (1.82)	4.39 (1.80)	.83
MEDAS score	5.0 (1.37)	4.86 (1.40)	.46
Restrictive diet[a]	30 (14)	8 (4)	5.90e-05
Bowel habit subtype, n (%)			
IBS-C	30 (28)		
IBS-D	41 (39)		
IBS-M	10 (9)		
IBS-U	25 (23)		
Overall severity (0–20)	10.11 (4.12)		
Abdominal pain (0–20)	8.94 (4.33)		
Bloating (0–20)	11.39 (5)		
VSI score (0–75)	39.69 (15.04)		
IBS-SSS (0–500)	248.91 (81.44)		

aMED, alternate Mediterranean index; BMI, body mass index; HADS, Hospital Anxiety and Depression Scale; HCs, healthy control subjects; IBS, irritable bowel syndrome; IBS-C, constipation-predominant IBS; IBS-D, diarrhea-predominant IBS; IBS-M, IBS with mixed bowel habits; IBS-U, IBS unclassified; IBS-SSS, Irritable Bowel Syndrome Severity Scoring System; MEDAS, Mediterranean Diet Adherence Score; VSI, Visceral Sensitivity Index.
[a]Gluten-free, dairy-free, and/or low FODMAP diets.

Table 2. Correlation Between MD Adherence and IBS Symptoms

	aMED Scores			MEDAS Scores		
	Estimate	Standard error	*P* value	Estimate	Standard error	*P* value
Overall symptoms	-0.08	0.24	.74	-0.12	0.32	.71
Abdominal pain	-0.03	0.25	.92	-0.39	0.33	.24
Bloating	0.37	0.28	.19	0.61	0.37	.10
VSI score	1.49	0.83	.08	1.17	1.05	.29
IBS-SSS	2.70	4.70	.57	3.06	6.17	.62

NOTE. The values were generated using linear regression covarying for body mass index, race, and Hospital Anxiety and Depression Scale-Anxiety. The estimates are beta values from the linear regression model.
aMED, alternate Mediterranean index; IBS, irritable bowel syndrome; IBS-SSS, Irritable Bowel Syndrome Severity Scoring System; MD, Mediterranean diet; MEDAS, Mediterranean Diet Adherence Score; VSI, Visceral Sensitivity Index.

margarine was associated with higher bloating and IBS-SSS scores (all $P < .05$) (Table 4).

The sparse partial least squares model identified pro-MD and anti-MD foods most associated with IBS symptoms to create a single dietary signature. Supplementary Figure 1 shows the loadings of the variables selected by the model. There was a negative correlation between overall dietary signatures scores and IBS symptoms (eg, dietary signature vs IBS-SSS, r = -0.46, P = .0001). Increased consumption of some anti-MD foods (eg, soda, processed meat, baked goods, and beer) was most associated with less IBS symptoms, whereas increased consumption of some pro-MD foods (eg, cantaloupe, carrot juice, grapefruit, sweet potato, and oranges/tangerines/clementines) was most associated with more IBS symptoms (Figure 1).

Our study showed that there was no difference in beta diversity between subjects with high, medium, and low aMED-m scores. However, a higher aMED-m score was associated (q-value <0.05) with lower abundance of *Faecalitalea*, *Streptococcus*, and *Intestinibacter*, and higher abundance of *Holdemanella* from the Firmicutes phylum (Figure 2).

Discussion

Our study showed that there was no difference in adherence to the MD between IBS participants and HCs. In addition, although MD was associated with higher diet quality, we did not find correlations between MD adherence and IBS symptoms. However, when the MD

Table 3. Differences in Consumption of Pro-Mediterranean and Anti-Mediterranean Food Groups Between IBS and HCs[a]

	IBS (N = 106)	HCs (N = 108)	Estimate	Standard error	FDR *P* value
Pro-MD foods, *g/d* (SD)					
Beans/legumes/soy	30.18 (63.88)	37.89 (69.34)	-0.015	0.005	.048
Fish/seafood	24.37 (32.41)	31.86 (52.93)	-0.012	0.005	.12
Fruit	262.8 (278.14)	263.9 (313.92)	-2.6e-04	0.001	.70
Grains (whole)	102.74 (105.86)	104.8 (124.56)	8.4e-05	0.001	.95
Nut/seeds	19.59 (30.46)	19.21 (24.75)	-0.003	0.006	.70
Olive oil	3 (3.1)	3.57 (4.37)	-0.08	0.051	.26
Poultry	49.77 (62.8)	48.24 (70.33)	-0.004	0.003	.32
Vegetables	248.37 (256.15)	308.95 (417.42)	-0.001	0.001	.12
Water	2350.04 (2603.21)	2329.08 (2749.07)	-7.5e-05	6.5e-05	.39
Wine	21.81 (30.53)	25.05 (36.96)	-0.007	0.006	.39
Anti-MD foods, *g/d* (SD)					
Added sugar	253.86 (590.78)	269.28 (806.63)	-0.0002	0.0002	.98
Alcohol (not wine)	105.93 (225.17)	68.12 (110.58)	0.001	0.001	.64
Baked goods/dessert/sweets	26.73 (34.07)	43.96 (88.02)	-0.008	0.004	.64
Butter, margarine, cream	23.58 (68.92)	18.62 (35.99)	-0.004	0.004	.92
Fried food	19.29 (21.47)	25.28 (42.51)	-0.01	0.006	.64
Red/processed meat	54.25 (69.84)	80.68 (148.31)	-0.003	0.002	.64

NOTE. The values were generated using generalized linear model (link=logit) covarying for body mass index, race, and Hospital Anxiety and Depression Scale-Anxiety. The estimates are beta values from the model.
FDR, false discovery rate; HCs, healthy control subjects; IBS, irritable bowel syndrome; MD, Mediterranean diet; SD, standard deviation.
[a]Consumption is measured in average total grams/day consumed per subject (SD).

Table 4. Correlation Between Mediterranean Food Intake and IBS Symptoms

	Overall symptoms		Abdominal pain		Bloating		VSI		IBS-SSS	
Pro-MD foods	Estimate	*P* value	Estimate	*P* value	Estimate	*P* value	Estimate	*P* value	Estimate	*P* value
Beans/legume/soy	-0.05	.0004	-0.023	.12	-0.018	.29	-0.061	.21	-0.941	.002
Fish/seafood	0.011	.47	0.022	.17	0.015	.43	0.093	.08	0.335	.28
Fruit	0.003	.10	0.004	.009	0.006	.004	0.007	.17	0.101	.0005
Grains (whole)	-0.003	.51	-0.001	.87	-0.007	.16	0.016	.25	-0.1	.20
Nut/seeds	-0.001	.95	0.003	.83	0.006	.75	-0.019	.72	0.115	.69
Olive oil	0.129	.39	0.183	.23	0.235	.18	0.051	.92	3.054	.27
Poultry	0.001	.85	0.003	.71	0.001	.88	0.014	.57	-0.006	.97
Vegetables	0.001	.48	0	.82	0.004	.072	0.013	.027	0.045	.18
Water	0	.74	0	.71	0	.40	0	.92	0.003	.36
Wine	0.012	.51	-0.006	.76	0.001	.48	0.076	.21	0.381	.22
Anti-MD foods										
Added sugar	0.001	.14	0.003	.006	0.001	.48	0.004	.21	0.033	.08
Alcohol (not wine)	-0.001	.67	0	.92	-0.004	.13	-0.013	.07	-0.066	.09
Baked goods/dessert/sweets	0.004	.78	0	.98	-0.017	.25	0.052	.24	-0.137	.58
Butter/margarine/cream	0.01	.25	0.005	.54	0.021	.03	0.041	.12	0.346	.02
Fried food	0.015	.45	0.011	.62	-0.013	.58	0.014	.84	0.012	.98
Red meat/processed meat	-0.001	.88	0.008	.23	-0.004	.66	0.012	.58	-0.103	.44

NOTE. This table shows the associations between Mediterranean food item intake and IBS symptom severity measures and the estimates represents the regression beta values generated using linear regression covarying for body mass index, race, and Hospital Anxiety and Depression Scale-Anxiety.
IBS, irritable bowel syndrome; IBS-SSS, Irritable Bowel Syndrome Severity Scoring System; MD, Mediterranean diet; VSI, Visceral Sensitivity Index.

was further analyzed by its main food groups, we found that a higher intake of certain pro-MD and anti-MD foods was associated with greater severity of IBS symptoms. Interestingly, multivariate analysis of individual food items showed that higher consumption of several pro-MD foods, such as cantaloupe, was associated with higher IBS symptoms, whereas higher consumption of several anti-MD foods, such as soda, was associated with lower IBS symptom severity.

Previous studies showed an inverse relationship between adherence to the MD and prevalence of IBS. Zito et al[14] surveyed 1134 participants in Southern Italy and found an association between lower adherence to MD and higher prevalence of IBS and functional dyspepsia in younger participants ($P < .05$). The authors concluded that low adherence to the MD may be a risk factor for development of disorders of gut-brain interaction in this population.[14] Similarly, Agakidis et al[15] studied 1116 children in Greece and found that good adherence to MD was associated with lower prevalence of disorders of gut-brain interaction, such as functional constipation, IBS, and functional dyspepsia according to the Rome III criteria ($P = .001$). In contrast, our study did not show a difference in MD adherence between IBS and HCs. This may be caused by differences in the study populations and standard diets. Furthermore, Agakidis et al[15] studied children from age 6–18 and the study by Zito et al[14] only showed significant association between MD adherence and IBS in the younger age group, whereas our study included only adults and was not stratified by age. It is possible that IBS participants in these studies consumed less MD foods that aggravated their symptoms; however,

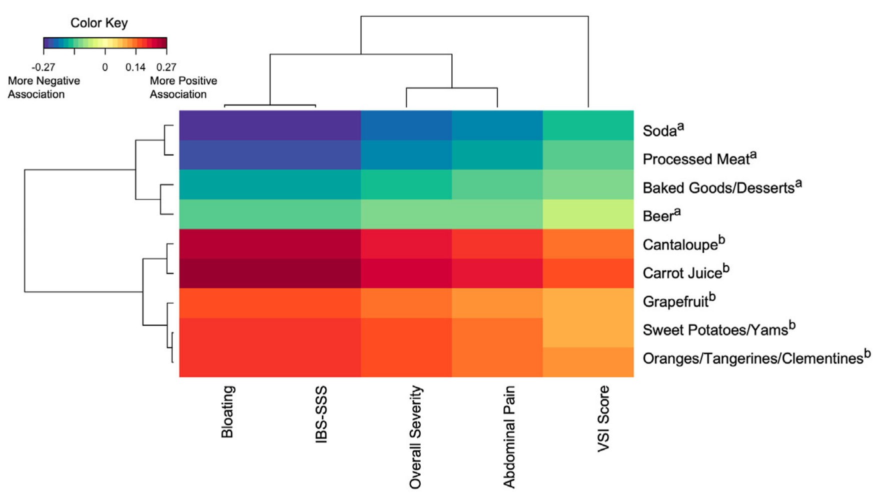

Figure 1. Sparse partial least squares analysis of the correlations between MD food items and IBS symptoms. Anti-MD foods are foods typically avoided or consumed occasionally in a Mediterranean style diet. Pro-MD foods are foods preferentially consumed in a Mediterranean-style diet. The heatmap shows Pearson correlation coefficients between the derived dietary variables and derived symptom variables. *Deeper red* color represents higher positive correlation and *deeper blue* represents higher negative correlation. Dendrogram row/column clusters based on the hierarchical clustering method. [a]Anti-MD foods. [b]Pro-MD foods.

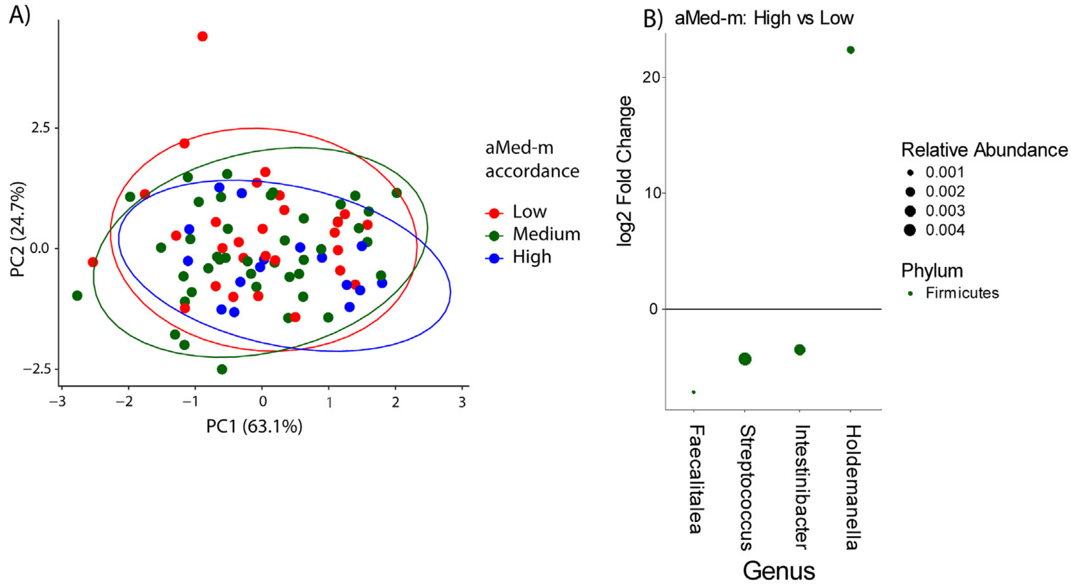

Figure 2. (*A*) Principal coordinate analysis comparison in beta-diversity between IBS subjects with low, medium, and high symptom aMED-m. (*B*) Relative abundance of fecal microbiota in IBS subjects with high aMED-m versus low aMED-m score.

the association between MD adherence and GI symptoms was not assessed in these studies.

More recently in 2021, Altomare et al[16] conducted a pilot study in Rome, Italy where dietary habits, IBS symptoms, and gut microbiome were compared between 28 IBS participants and 21 HCs. This study found that IBS participants had lower MD adherence score compared with HCs. There was no association between MD adherence and IBS symptoms of abdominal pain and flatulence. Specific microbial biomarkers were detected for altered and adequate nutrient intake in patients with IBS. These results agreed with our findings that MD adherence was not associated with IBS symptoms. However, this was a much smaller study with only 28 IBS participants compared with our larger study.

Previous studies have shown that adherence to the MD is associated with positive changes to the gut microbiome.[5] Similarly, our study showed that IBS subjects who were adherent to a symptom-modified MD were associated with a lower abundance of potentially proinflammatory, pathogenic, and gas-producing microbes, such as *Faecalitalea*, *Intestinibacter*, and *Streptococcus*,[28-30] and a higher abundance of anti-inflammatory *Holdemanella*[31] from the Firmicutes phylum. These preliminary findings suggest that symptom-modified MD may be associated with beneficial changes to the microbiome. However, future studies should assess microbial function because 16S rRNA analysis measures microbial composition but not function.

Despite being a healthy diet, the MD has not been shown to be beneficial for IBS symptom severity in our study and the literature. Prior studies on dietary management of IBS show that the low FODMAP diet and the National Institute for Health and Care Excellence traditional diet for IBS are effective in reducing IBS symptoms,[11,32] which recommend limited intake of FODMAPs, high-fiber foods, resistant starches, and fruits. In contrast, the main components of the MD include fruits, vegetables, whole grains, and legumes, many of which have been shown to be associated with increased IBS symptoms in our study. The pro-MD foods identified to increase IBS symptoms are also associated with higher quantities of FODMAPs. Therefore, these components of a standard MD are not considered to be a part of an IBS-friendly diet and should be reduced to a quantity that can be tolerated for those with more severe IBS symptoms. Similarly, individuals may have different food triggers, thus a generalized MD may not be suitable for all patients with IBS or needs to be personalized as with a low FODMAP diet.

In our study, IBS participants consumed less beans/legumes/soy compared with HCs likely because these foods can trigger GI symptoms. In IBS, the higher consumption of beans/legumes/soy was associated with lower IBS symptom severity but not GI symptom-related anxiety. It is possible that beans/legumes/soy were preferentially avoided in IBS participants with more severe symptoms and consumed more in those with relatively lower visceral sensitivity or less gas-producing gut microbiome. However, they do not seem to be reducing their consumption of beans/legumes/soy because of worries or fears related to IBS symptoms. Additionally, several anti-MD foods were also associated with less IBS symptoms. We previously showed that patients with IBS with more severe IBS symptoms consumed a more restrictive diet.[13] Thus, our findings might simply demonstrate that those with milder IBS can tolerate a greater variety of foods.

There were strengths that differentiated our study from prior studies. It is one of the largest studies to date that examined the association between MD intake and

IBS symptoms with 106 IBS participants and 108 HCs. Our study included all IBS bowel habit subtypes and controlled for covariates including age, race, and Hospital Anxiety and Depression Scale-Anxiety. We used validated MD adherence scores and performed a detailed dietary assessment of the MD including individual pro-MD and anti-MD food groups and food items. Moreover, IBS symptoms were assessed using multiple validated instruments.

There were several limitations. This study was a cross-sectional study; therefore, patients were not randomized into specific dietary interventions and the results only demonstrated association but not causation. In addition, diet was assessed with DHQ II, which relied on diet recall in the past year instead of multiple time points and did not capture lifestyle components of the MD. Furthermore, the population of this study was based in Los Angeles, which resulted in lower average MD scores compared with the Mediterranean populations. In addition, our population also consumed significantly less olive oil compared with MD studies (average of 3g vs 55g[3]). To bridge this gap, our study also included aMED score, which was developed to assess MD intake in non-Mediterranean countries and accounted for differences in dietary patterns in different populations. However, our population may still be consuming a different MD than those in Mediterranean countries.

In summary, our study showed that although a standard MD was a healthy diet, adherence to the MD was not higher in IBS participants compared with HCs, nor was it associated with less severe IBS symptoms. Certain pro-MD foods were associated with increased symptoms, possibly because of high FODMAP content. Patients with milder IBS symptoms may be more liberal with their diet, whereas those with more severe symptoms may need to restrict certain anti-MD foods to lessen symptoms. Our findings suggest that an IBS-modified MD, rather than a standard MD, should be considered to reduce IBS symptom severity in research studies and clinical practice.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2023.07.012.

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Conflicts of interest

These authors disclose the following: Emeran Mayer is a scientific advisory board member of Danone, Axial Biotherapeutics, Amare, Mahana Therapeutics, Pendulum, Bloom Biosciences, Seed, and APC Microbiome Ireland. Lin Chang is consultant for Food Marble; and has stock options in Food Marble and ModifyHealth. The remaining authors disclose no conflicts.

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Supplementary Methods

Irritable Bowel Syndrome Symptom-Related Questionnaires

Bowel Symptom Questionnaire. The Bowel Symptom Questionnaire[1] is a self-reported assessment that includes questions to evaluate Rome IBS symptoms and severity of IBS symptoms. The severity of abdominal pain, bloating, and overall symptoms are measured using numeric scales ranging from 0–20 with 0 meaning none and 20 meaning most severe pain, bloating, or overall symptoms.

Irritable Bowel Syndrome Severity Scoring System. The Irritable Bowel Syndrome Severity Scoring System[2] is a validated instrument that assesses the frequency and severity of abdominal pain, severity of abdominal distention, dissatisfaction with bowel habits, and interference of IBS with daily life scored from 0–100 in each of the 5 categories. The total Irritable Bowel Syndrome Severity Scoring System score is the sum of these categories with a total score range of 0–500.

Psychological Symptoms Assessment

Visceral Sensitivity Index. The Visceral Sensitivity Index[3,4] is a 15-item questionnaire used to measure gastrointestinal symptom-specific anxiety of patients with IBS. Each item is scored from 0–5 with 0 being strongly disagree to 5 being strongly agree. The total score ranges from 0–75 with 0 meaning no gastrointestinal-specific anxiety and 75 meaning severe gastrointestinal-specific anxiety.

Hospital Anxiety and Depression Scale. Hospital Anxiety and Depression Scale[5] is a 14-item self-assessment scale that is designed for assessing current states of anxiety and depression in a nonpsychiatric setting. Seven of the items are related to anxiety and 7 other items are related to depression. Each item is scored 0–3. The total score for anxiety and depression are both 0–21 with 0–7 being normal, 8–10 being borderline, and 11–21 being probably clinical anxiety or depression.

Dietary Assessment

Diet History Questionnaire II. The DHQ II[6] is a self-administered food frequency questionnaire. The frequency and quantities of 134 food items and 8 dietary supplement questions are measured based on dietary recall of the past 12 months. Gender-specific diet data, including food group intake estimates (grams/day), from the DHQ II are analyzed using the Diet*Calc software.

Alternate Mediterranean Index. The Alternate Mediterranean Index[7] score includes 9 dietary components that are characteristic for an MD. It takes into consideration differences in dietary patterns in a population, and each item is scored based on comparison with population median. The Alternate Mediterranean Index score is associated with significantly lower concentrations of inflammatory biomarkers. Components of the Alternate Mediterranean Index score are found in Supplementary Table 1. The score is ranged from 0–9 where low accordance tertile ranged from 0–3, the medium accordance tertile scored 4–6, and the high accordance tertile ranged from 7–9.

Mediterranean Diet Adherence Screener. The MEDAS[8] score was developed for assessment of MD adherence for the Prevención con Dieta Mediterránea (PREDIMED) study, which showed that participants with a high risk of cardiovascular disease were able to lower that risk after adherence to an MD high in extravirgin olive oil and nuts compared with those who followed a reduced-fat diet.[9] Higher MEDAS scores were associated with higher high-density lipoprotein/cholesterol and lower body mass index, waist circumference, triglyceride, triglyceride/high-density lipoprotein ratio, fasting glucose, and cholesterol/high-density lipoprotein/cholesterol ratio. Components and calculations of the MEDAS score are found in Supplementary Table 2. The maximum for MEDAS score was 14; however, when converting DHQ items to MEDAS score, we were unable to translate Sofrito intake from the DHQ, and therefore, the maximum score in our population was 13. In previous studies, good or very good adherence to MD diet correlated to a MEDAS score of $\geq$10.

Alternative Healthy Eating Index-2010. Alternative Healthy Eating Index awards up to 10 points for each of the 11 food groups and nutrients predictive of chronic disease with a maximum score of 110. Higher Alternative Healthy Eating Index scores have been shown to be strongly associated with a lower risk of major chronic diseases, coronary heart disease, diabetes, and stroke.[10,11] Components of the Alternative Healthy Eating Index-2010 score are found in Supplementary Table 3.

Model Specification and Tuning Parameters for Building Spatial Partial Least Squares Models

Sparse partial least squares (sPLS-R)-regression[12] implemented in mixOmics R package[13] was used to determine the relationships between dietary intake of anti-MD and pro-MD foods with IBS symptom severity measures. This latent variable approach uses simultaneous data integration and variable selection via LASSO penalization that maximizes the covariance between sets of data (ie, linear combination of data type reflecting a dietary or symptom profiles or pattern). Visualization of the results is provided in the form of loading plots that reflect the contribution of each variable to each profile with variable with the highest loading having the highest influence on the derived profiles. Furthermore, cluster image maps are provided to show the Pearson correlation coefficients between the variable in the matched profiles. As a part of the cluster image map dendrograms are used along the axes to depict how each row/column clusters based on the hierarchical clustering method.

The tuning parameters (ie, the number of components and the number of variables in dietary variables [keepX] and IBS symptom variables [keepY] to select) were tuned using "tune.spls" function in mixOmics R package.[13] M-fold cross-validation with validation with 10-folds and 10 repeats was performed and accuracy measure "mean square error calculated" was calculated. Based on the output of the tuning function, 2 components were selected as the optimal number of components, 9 dietary variables selected as optimal number of keepX variables, and all symptom variables were included as keepY variables. Model was trained using the tuning parameters using "spls" function with a "regression" mode to identify select subsets of variables from both data sets that are highly positively or negatively correlated across samples. Pearson correlation was performed to assess the correlation between the dietary and symptom signature. Performance was evaluated using "perf" function using M-fold cross-validation with 5 folds and 10 repeats.

Multivariate models, such as partial least squares (PLS), are well-suited to situations where there are multiple predictor and/or response variables. Unlike principal component analysis, PLS models are more robust to highly correlated features. Additionally, sPLS models use "lasso" penalization, which forces the coefficients of "unimportant" features to be zero effectively eliminating them from the model, which aids in better feature selection. This results in a simpler more interpretable model. To test a correlation structure within our predictors, we used Pearson correlation coefficients to test whether there was a negative correlation between pro-MD and anti-MD foods in the selected features. We did not find any notable negative correlations between pro-MD and anti-MD foods that can explain the observed relationship between increased consumption of pro-MD foods (cantaloupe, carrot juice, grapefruit, sweet potato, oranges/tangerines/clementine) and IBS symptoms or decreased consumption of anti-MD.

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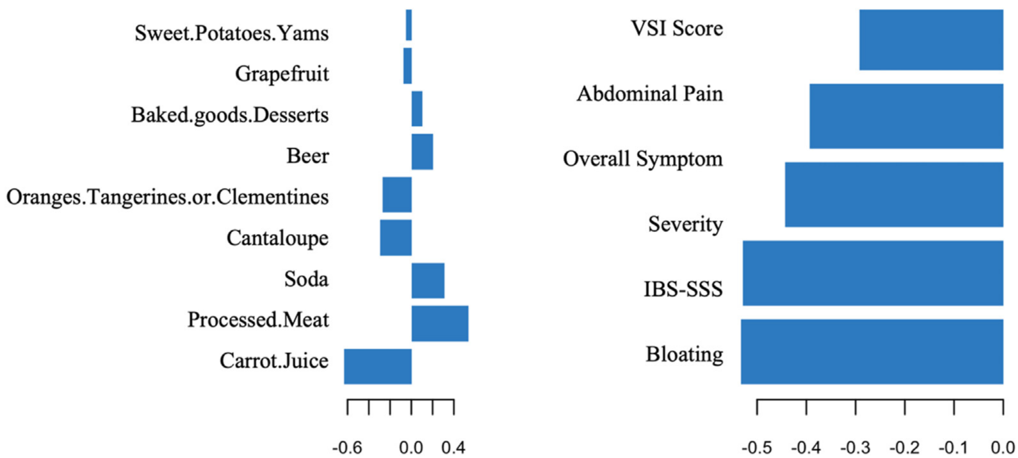

Supplementary Figure 1. Loadings plot of dietary and IBS symptom variables for sPLS model. Tuning the sPLS model to test the performance of a 1-component versus 2-component model resulted in a better performance (ie, a higher correlation between the Mediterranean diet items and IBS symptoms) for a 1-component system. This figure shows the loadings of the variables selected by the sPLS model to be significantly associated food items and IBS symptoms. The *horizontal bar plots* visualize loading vectors where each bar length corresponds to the loading weight (importance) of the feature.

Supplementary Table 1. Alternate Mediterranean Diet Score Components and Criteria for Scoring

Food group	Foods included	Criteria for 1 point
Vegetables	All vegetables except potatoes	Greater than median intake (servings/d)
Legumes	Tofu, string beans, peas, beans	Greater than median intake (servings/d)
Fruit	All fruit and juices	Greater than median intake (servings/d)
Nuts	Nuts, peanut butter	Greater than median intake (servings/d)
Whole grains	Whole-grain ready-to-eat cereals, cooked cereals, crackers, dark breads, brown rice, other grains, wheat germ, bran, popcorn	Greater than median intake (servings/d)
Red and processed meats	Hot dogs, deli meat, bacon, hamburger, beef	Less than median intake (servings/d)
Fish	Fish and shrimp, breaded fish	Greater than median intake (servings/d)
Ratio of monounsaturated to saturated fat	—	Greater than median intake (servings/d)
Ethanol	Wine, beer, "light" beer, liquor	Women: 5–25 g/d Men: 10–50 g/d

Supplementary Table 2. Mediterranean Diet Adherence Screener Score Components and Criteria for Scoring

Foods and frequency of consumption	Criteria for 1 point[a]
1. Do you use olive oil as main culinary fat?	Yes
2. How much olive oil do you consume in a given day (including used for frying, salads, out of house meals)?	4 or more tablespoons
3. How many vegetable servings do you consume per day (1 serving = 200 g, consider side dishes as half serving)	2 or more (at least 1 portion raw or as salad)
4. How many fruit units (including natural fruit juices) do you consume per day?	3 or more
5. How many servings of red meat, hamburger, or meat products (eg, ham, sausage) do you consume per day? (1 serving = 100–150 g)	Less than 1
6. How many servings of butter, margarine, or cream do you consume per day? (1 serving = 12 g)	Less than 1
7. How many sweet/carbonated beverages do you drink per day?	Less than 1
8. How much wine do you drink per week?	7 or more glasses
9. How many servings of legumes do you consume per week? (1 serving = 150 g)	3 or more
10. How many servings of fish or shellfish do you consume per week? (1 serving: 100–150 g fish, or 4–5 units or 200 g shellfish)	3 or more
11. How many times per week do you consume commercial sweets or pastries (not homemade), such as cakes, cookies, biscuits, or custard?	Less than 3
12. How many servings of nuts (including peanuts) do you consume per week? (1 serving = 30 g)	3 or more
13. Do you preferentially consume chicken, turkey, or rabbit meat instead of veal, pork, hamburger, or sausage?[b]	Yes
14. How many times per week do you consume vegetables, pasta, rice, or other dishes seasoned with sofrito (sauce made with tomato and onion, leak, or garlic, simmered in olive oil)?	2 or more

[a]0 points if these criteria are not met.
[b]1 point for vegetarians.

Supplementary Table 3. Alternative Healthy Eating Index-2010 Components and Criteria for Scoring

Component	Criteria for a minimum score of 0	Criteria for maximum score of 10
Whole fruit	0	≥4 servings/d
Vegetables (excluding potatoes)	0	5 servings/d
Whole grains	0	Women: 75 g/d Men: 90 g/d
Red and processed meat	≥ 1.5 servings/d	0
Nuts and legumes	0	1 serving/d
Long-chain (ω-3) fats and (EPA+DHA)	0	250 mg/d
Polyunsaturated fatty acids		10% of energy
Trans fat	≥4% of energy	
SSBs and fruit juice	1 serving/d	0
Sodium	Highest decile	Lowest decile
Total	0	100

DHA, docosahexaenoic acids; EPA, eicosapentaenoic acids; SSB, sugar sweetened beverage.

Supplementary Table 4. Full List of Pro-MD and Anti-MD Foods

	Food item
Pro-MD food groups	
Wine	Wine
Beans/legumes/soy	Beans
	Soy
Fruits	Orange or grape juice
	Other fruit juice
	Applesauce
	Apples
	Pears
	Bananas
	Dried fruit
	Peaches, nectarines, or plums
	Grapes
	Cantaloupe
	Melon other than cantaloupe
	Strawberries
	Oranges, tangerines, or clementines
	Grapefruit
	Pineapple
	Other fruits
Fish/seafood	Salmon, tuna, or trout
	Other fish not fried
	Shellfish not fried
Grains (whole)	Oatmeal, grits, or other cooked cereal
	High-fiber cereal
	Rice or other cooked grains
	Whole wheat/grain bread
Nut/seeds	Peanut or nut butters
	Peanuts, walnuts, seeds, or other nuts
Olive oil	Olive oil
Poultry	Turkey or chicken cold cuts
	Ground chicken or turkey
	Roast turkey
	Chicken mixtures
	Chicken
Vegetables (not corn/potato)	Carrot juice
	Tomato juice or other vegetable juice
	Cooked greens
	Raw greens
	Carrots
	String beans
	Peas
	Broccoli
	Cauliflower or Brussels sprouts
	Asparagus
	Winter squash
	Mixed vegetables
	Onions
	Sweet peppers
	Fresh tomatoes
	Lettuce salads
	Sweet potatoes/yams
	Other kinds of vegetables
Water	Water
Anti-MD food groups	
Alcohol (not wine)	Beer
	Liquor/mixed drinks
Processed/red meat	Processed meat
	Beef
	Pork or beef spareribs
	Pork
Added sugar	Soda
	Sport/energy drink
	Sugar added to foods
Baked goods/dessert/sweets	Frozen yogurt, sorbet, or ices
	Baked goods/desserts
	Chocolate candy
	Other candy
Butter/margarine/cream	Butter, margarine, cream
Fried food	French fries
	Fried shellfish
	Fried fish
	Chips

MD, Mediterranean diet.