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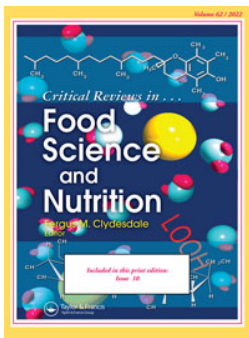
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## Effects of a mediterranean diet on the gut microbiota and microbial metabolites: A systematic review of randomized controlled trials and observational studies

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# Effects of a mediterranean diet on the gut microbiota and microbial metabolites: A systematic review of randomized controlled trials and observational studies

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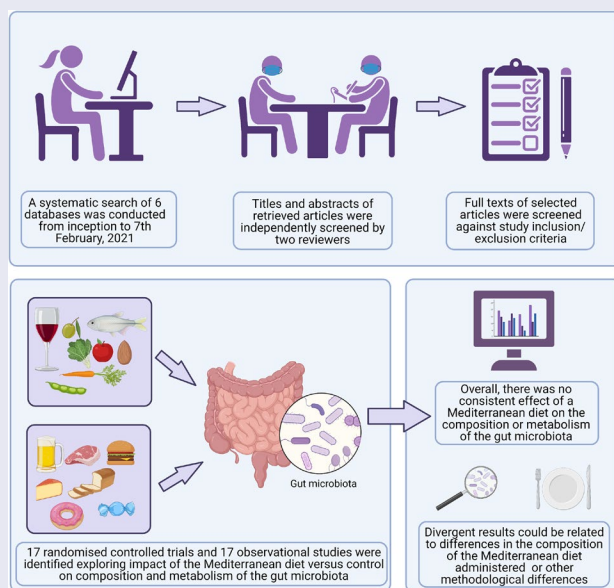
## ABSTRACT

Consumption of the Mediterranean dietary pattern (MedDiet) is associated with reduced risk of numerous non-communicable diseases. Modulation of the composition and metabolism of the gut microbiota represents a potential mechanism through which the MedDiet elicits these effects. We conducted a systematic literature search (Prospero registration: CRD42020168977) using PubMed, The Cochrane Library, MEDLINE, SPORTDiscuss, Scopus and CINAHL databases for randomized controlled trials (RCTs) and observational studies exploring the impact of a MedDiet on gut microbiota composition (i.e., relative abundance of bacteria or diversity metrics) and metabolites (e.g., short chain fatty acids). Seventeen RCTs and 17 observational studies were eligible for inclusion in this review. Risk of bias across the studies was mixed but mainly identified as low and unclear. Overall, RCTs and observational studies provided no clear evidence of a consistent effect of a MedDiet on composition or metabolism of the gut microbiota. These findings may be related to the diverse methods across studies (e.g., MedDiet classification and analytical techniques), cohort characteristics, and variable quality of studies. Further, well-designed studies are warranted to advance understanding of the potential effects of the MedDiet using more detailed examination of microbiota and microbial metabolites with reference to emerging characteristics of a healthy gut microbiome.

## KEYWORDS

Gut microbiota;  
Healthy aging;  
Mediterranean diet;  
Short-chain fatty acids

## GRAPHICAL ABSTRACT



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This systematic review explored effects of the Mediterranean diet on the composition and metabolism of the gut microbiota in both randomized controlled trials and observational studies. Although there is some evidence from a small number of studies indicated a positive impact of a Mediterranean diet on select microbiota, the findings of this systematic review suggest that this dietary pattern does not consistently alter microbiota composition or metabolism. The lack of a consistent effect could be related to methodological differences between studies, especially differences in the composition of the Mediterranean diet administered.

## Introduction

Over the last few decades, the gut microbiome (a highly complex and diverse community of microbes living within gastrointestinal tract) has received considerable attention because of its fundamental role in human health and disease (Valdes et al. 2018). There is growing evidence that the gut microbiota plays a critical, symbiotic role in human health, participating in, amongst others, vitamin biosynthesis, the fermentation of undigested carbohydrates and proteins to produce short chain fatty acids (SCFAs; e.g., acetate, propionate and butyrate), the maintenance of gastrointestinal integrity, and regulation of immune function (Hooper and Gordon 2001; Rowland et al. 2018; Valdes et al. 2018). In addition, disturbances in the gut microbiota (i.e., dysbiosis) have been linked with a multitude of adverse health outcomes including inflammatory bowel disease, colorectal cancer, diabetes, obesity, cardiovascular disease, and dementia (Hills et al. 2019). The gut microbiota is modulated by diet, and also by other lifestyle factors including physical activity (PA), and smoking (Valdes et al. 2018), which have bigger effects than host genetic factors (Rothschild et al., 2018). Diet plays a significant role in shaping the microbiome by providing substrates that can differentially promote the growth and activities of specific microbes and communities (Singh et al. 2017) with, potentially, consequential beneficial effects on health (Hills et al. 2019; Valdes et al. 2018).

The Mediterranean dietary pattern (MedDiet), which reflects the traditional eating habits in countries situated around the Mediterranean Sea prior to the globalization of food systems, is a plant-based dietary pattern rich in fruit, vegetables, nuts, seeds, olive oil, and unrefined grains. Small-to-moderate amounts of animal products are typically consumed as part of the MedDiet, including moderate quantities of fish, small amount of poultry, and minimal consumption of red and processed meats. In addition, the MedDiet typically includes a low-to-moderate intake of alcohol, usually in the form of red wine, which is consumed socially at meal times (Bach-Faig et al. 2011; Shannon et al. 2019). Many of the characteristic components of the MedDiet including plant foods and red wine are rich in nutrients such as fiber and in phytochemicals (e.g. polyphenols) that have been independently associated with reduced risk of mortality (Kim & Je, 2017; Zamora-Ros et al., 2013) and morbidity from non-communicable diseases (Reis et al., 2016; Shishtar et al., 2020; Threapleton et al., 2013). Dietary fiber and polyphenols are known to have prebiotic actions which might mediate health-related outcomes associated

with their intake (Gibson et al. 2017; Tuohy et al. 2012). Dietary fiber is fermented by bacteria in the colon to produce SCFA which are proposed to have systemic anti-inflammatory effects (van der Hee and Wells 2021). Polyphenols are metabolized initially by the gut microbiota, resulting in increased absorption, and the subsequent metabolism by host enzymes in the gut mucosa and liver can produce metabolites with multiple physiological effects (Istas et al., 2019). In addition, some polyphenols act as antimicrobials against pathogenic bacteria with consequential effects on host health (Ma & Chen, 2020).

While consumption of individual foods and food components may have metabolic and health consequences, they are not eaten in isolation and so to understand the overall effects of dietary choices it is important to characterize dietary patterns and to investigate their links with health (Schulze et al. 2018). The complex array of nutrients and other bioactive components within a dietary pattern, such as the MedDiet, will result in interactions and synergies that may influence the gut bacteria (Riaz Rajoka et al. 2017) in multiple ways. However, evidence from individual studies is often conflicting and paradoxical. For example, the diversity of the gut microbiota has been reported to increase in some studies (Cox et al. 2020; Maskarinec et al. 2019) and not change in others (Maldonado-Contreras et al. 2020; Meslier et al. 2020) with higher adherence to MedDiet. Similarly, the relative abundance of bacterial phyla (e.g., Bacteroidetes) has been shown to increase with greater MedDiet adherence, but decrease after MedDiet intervention (Di Iorio et al. 2019; Gutiérrez-Díaz et al. 2016). Therefore, we aimed to conduct the first systematic review exploring effects of the MedDiet on the composition and metabolism of the gut microbiota, drawing evidence from both observational studies and RCTs.

## Methods

This systematic review was conducted according to the Preferred Reporting Items for Systematic Review and Meta-analyses (PRISMA) guidelines (Liberati et al. 2009) and was registered prospectively with the PROSPERO database (CRD42020168977).

### Literature search

A systematic search of six databases (PubMed, The Cochrane Library, MEDLINE, SPORTDiscuss, Scopus and CINAHL)

was conducted from inception through to 7<sup>th</sup> February 2021 to identify relevant articles for inclusion in this review. No publication date or language restrictions were applied. Searches were conducted using pre-defined search terms relating to the MedDiet and the composition and metabolism of the gut microbiota, with MeSH terms utilized where appropriate. The search strategy was tailored to the requirements of each database (Supplementary Material 1). The reference lists of eligible studies and relevant review articles were also searched to identify other potentially relevant studies. Gray literature was included in search results to minimize risk of publication bias.

### Study selection

#### Inclusion criteria

The following criteria were used to identify appropriate articles for inclusion in this systematic review:

#### General criteria

1. RCTs and observational studies were included. No exclusion criteria were applied based around the study design for either RCTs (e.g. cross-over or parallel group design) or observational studies (e.g. cross-sectional, case-control, or prospective studies).
2. Studies with adult participants (aged  $\geq 18$  years) were included. Participants were not excluded based on health or smoking status.

#### Study-specific criteria

##### Observational studies

1. Studies exploring associations between MedDiet adherence and the composition and/or metabolism of the bacterial community were included, providing that MedDiet adherence was compared against a suitable reference (e.g., low MedDiet adherence) or control (e.g., intake of an alternative dietary pattern) group.
2. No exclusion criteria were made based on the method used to define MedDiet adherence.

##### RCTs

3. RCTs which explored the effects of a MedDiet intervention alone or in combination with other clinical, pharmacological or lifestyle interventions were included, as long as the study also included a suitable control group. For example, if the MedDiet was administered in combination with an exercise intervention, the study was within scope if the control group also underwent the same exercise intervention.
4. No exclusion criteria were made based on the specific composition of the MedDiet; we included articles where the dietary intervention was described as MedDiet by the authors of the article.

### Exclusion criteria

We excluded studies that included non-adult subjects and studies including animals and in vitro studies. Studies were also excluded if the MedDiet was administered alongside another intervention (i.e., exercise, pharmacological agent, dietary supplement) and no suitable control or reference groups could be identified. For RCTs, use of antibiotics and/or laxatives before, or during, the intervention was an exclusion criterion, given their impact on the gut microbiota.

### Screening

The titles and abstracts of retrieved articles were independently screened by two researchers (PG and RK) to evaluate their eligibility for inclusion in this review. The researchers met after completing the independent review of titles/abstracts to compare notes and reach a consensus. A third reviewer (OMS) was present to moderate the discussion and resolve disagreements about the eligibility of potential studies. Potentially eligible studies, that could not be excluded from an appraisal of their titles and abstracts, were carried over to the full-text stage of the review for further evaluation. Full-texts of the selected articles were evaluated against the study inclusion/exclusion criteria by two researchers (PG and RK) and a third researcher (OMS) helped resolve any disagreements.

### Data extraction

Data were extracted independently by two reviewers (RK and PG) and were checked by a third reviewer (OMS). The following information was extracted: Surname of the first author, author contact details, publication year, country, information on the study design, study duration, study cohort, sample size, type of intervention (RCTs) and control group, method used to evaluate dietary intake and MedDiet adherence, age, gender, ethnicity, randomization procedure, blinding of measurements, compliance with the interventions, body mass index (BMI), medication use, smoking status, gut microbiota composition and times of measurements (e.g. baseline, post-intervention), markers of gut microbiota metabolism (e.g. SCFA concentrations) and the methods and techniques used to assess these measures.

### Data synthesis

The extracted data were deemed unsuitable for meta-analysis due to the diversity in outcome measurements and the small number of studies reporting data on the same microbiota-related outcomes (Jackson and Turner 2017). Therefore, a narrative synthesis of the literature was conducted for the effect of MedDiet on microbiota diversity, bacterial abundance as well as microbial metabolites (Li et al. 2018; Tomas-Barberan, Selma, and Espín 2018; Vernocchi, Del Chierico, and Putignani 2016).

## Assessment of study quality

Risk of bias of the included studies was assessed by one researcher (AG) and checked for accuracy by a second researcher (OMS). Study quality for RCTs was assessed using The Cochrane risk of bias tool (Higgins and Green 2011). Each RCT was classified as high, low or unclear (i.e. insufficient evidence) risk in relation to seven domains: sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other sources of bias. The Newcastle-Ottawa tool was used to assess risk of bias in observational studies. Studies were classified as high-quality ( $\geq 7$  stars), medium-quality (4-6 stars) or low quality (0-3 stars) (Islam et al. 2016; Wells et al. 2000). An adapted version of this tool was used to assess risk of bias in cross-sectional studies (Modesti et al. 2016).

## Results

### Overview

A total of 1249 non-duplicated records were identified following electronic database searches. After screening the titles and abstracts, 42 of the records were deemed potentially eligible for inclusion and full-texts were retrieved for further

evaluation. Two additional full-texts were identified via other sources and that were deemed potentially eligible for inclusion in this review. Following appraisal of 44 full-texts, a total of 34 articles (17 RCTs and 17 observational studies) were identified as eligible for inclusion in this review (Figure 1).

### Study characteristics

#### Observational studies

The characteristics of eligible observational studies are outlined in Table 1. Of the 17 observational studies, 14 were cross-sectional and 3 were prospective studies. The total number of participants across the observational studies was 4526. Based on the mean/median values provided in the studies, there was a median sample of 119 (range: 20-1735) and age of 60 (range: 22-95) years.

In the majority of studies, the impact of the MedDiet was investigated in healthy participants ( $n=10$ ) (Barrea et al. 2019; Filippis et al. 2016; Gallè et al. 2020; Garcia-Mantrana et al. 2018; Gutiérrez-Díaz et al., 2017; Gutiérrez-Díaz et al. 2016; Maskarinec et al. 2019; Mitsou et al. 2017; Valeriani et al. 2020; Wang et al. 2021). Studies were also conducted in individuals with atrial fibrillation ( $n=1$ ) (Pastori et al. 2017), diabetes ( $n=1$ ) (Pignanelli et al. 2018), diabetes, hypertension

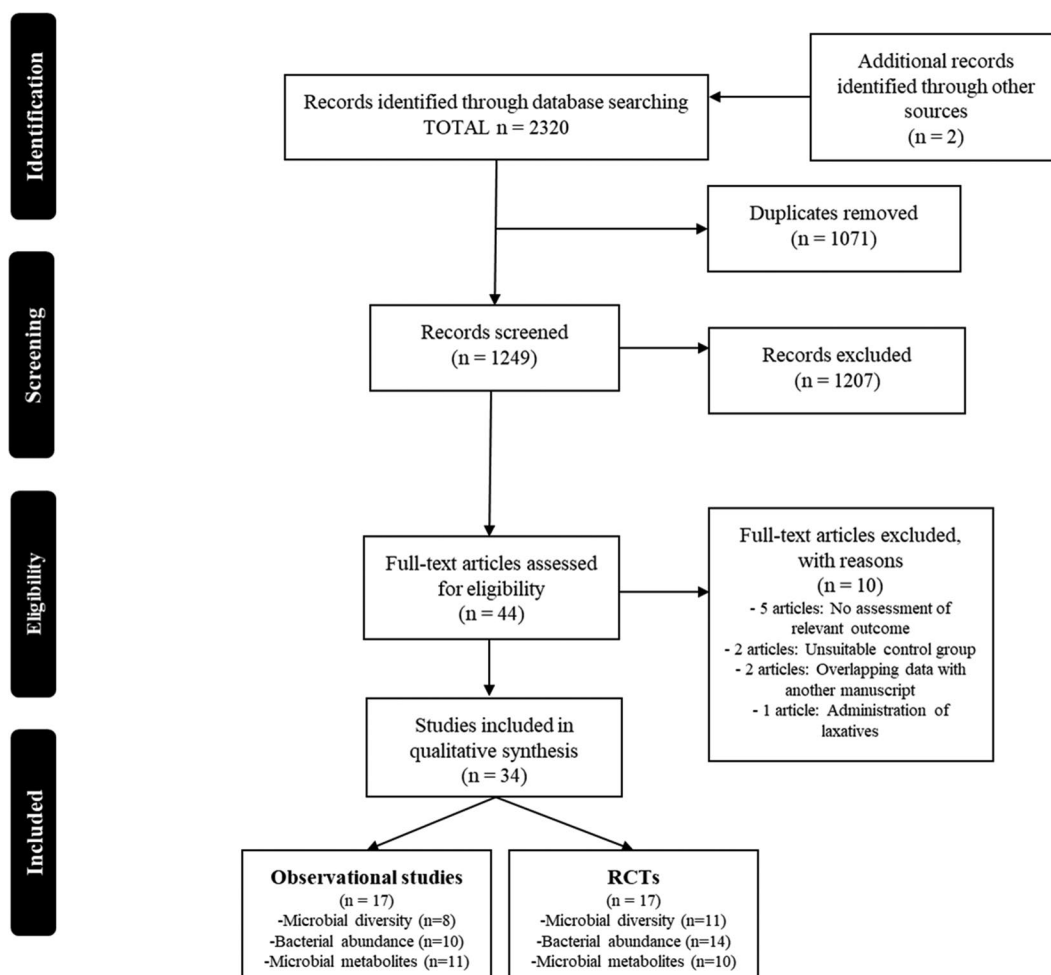


Figure 1. PRISMA flow diagram of studies included in this systematic review.

Table 1. Characteristics of observational studies investigating associations between MedDiet adherence and the gut microbiota.

Authors	Country	Study design	Health status	Sample size (n)	Male (n)	Age (y)	BMI (kg/m <sup>2</sup> )	Duration (months)	MedDiet score
Almanza-Aguilera et al. (2017)	Spain	Cross-sectional	Diabetes, Hypertension, and Hypercholesterolemia	119	37	67	30	N/A	14-point MEDAS score
Barrea et al. (2019)	Italy	Cross-sectional	Healthy	144	67	32	23	N/A	14-point MEDAS score
Cox et al. (2020)	USA Turkey	Cross-sectional	Cirrhosis	296	200	58	27	N/A	Not specified
De Filippis et al. (2016)	Italy	Cross-sectional	Healthy	153	64	27-47	22	N/A	11-item adapted Trichopoulou score
Picchianti Diamanti et al. (2020)	Italy	Cross-sectional	Rheumatoid arthritis	60	10	61	24	N/A	14-point MEDAS score
Galle et al. (2020)	Italy	Cross-sectional	Healthy	140	68	22	22	N/A	9-point MEDAS score
García-Mantrana et al. (2018)	Spain	Cross-sectional	Healthy	27	11	40	23	N/A	14-point MEDAS score
Gutiérrez-Díaz et al. (2016)	Italy	Cross-sectional	Healthy	31	8	42	26	N/A	8-point adapted Trichopoulou score
Gutiérrez-Díaz et al. (2017)	Spain	Cross-sectional	Healthy	74	20	71	–	N/A	8-point adapted Trichopoulou score
Maldonado-Contreras et al. (2020)	USA	Cross-sectional	High prevalence of chronic disease	20	6	63	–	N/A	9-point Trichopoulou score
Maskarinec et al. (2019)	USA	Prospective	Healthy	1735	858	69	28	240	8-point adapted Trichopoulou score
Mitsou et al. (2017)	Greece	Cross-sectional	Healthy	100	48	41	27	N/A	11-point Panagiotakos MedDiet score
Pastori et al. (2017)	Italy	Prospective	Atrial fibrillation	912	521	74	28	21-68	9-point MEDAS score
Pignatelli et al. (2018)	UK Canada	Cross-sectional	Diabetes	276	164	67	28	N/A	8-point Trichopoulou Score
Ruiz-Saavedra et al. (2020)	Spain	Cross-sectional	Non-declared pathologies	73	20	56-95	20-38	N/A	9-point Trichopoulou Score
Valeriani et al. (2020)	Italy	Cross-sectional	Healthy	59	29	23	22	N/A	9-point MEDAS score
Wang et al. (2021)	USA	Prospective	Healthy at baseline	307	307	45-80	–	324 <sup>†</sup>	9-point adapted Trichopoulou score

MEDAS = Mediterranean diet adherence screener, † = 324 months between enrollment in 1986 and last sample collection in 2013.

and hypercholesterolemia (n=1) (Almanza-Aguilera et al., 2017), cirrhosis (n=1) (Cox et al. 2020), rheumatoid arthritis (n=1) (Picchianti Diamanti et al. 2020), non-declared pathologies (n=1) (Ruiz-Saavedra et al. 2020), and a high prevalence of chronic disease (n=1) (Maldonado-Contreras et al. 2020). A range of instruments were used to characterize MedDiet adherence, which are summarized in Table 1 and described in detail in Supplementary Table 4.

### **Randomized controlled trials**

The characteristics of eligible RCTs are outlined in Table 2. Of the 17 RCTs, 12 used parallel group, and 5 used cross-over, study designs. The total number of participants in the RCTs was 1882. Based on the mean/median values provided in the studies, there was a median sample size of 79 (range: 10-612), age of 60 (range: 22-79) years and intervention duration of 26 (range: <1-156) weeks. Participants included healthy individuals (n=2) (Park et al. 2019; Zhu et al. 2020), healthy participants at increased risk of colon cancer (n=3) (Djuric et al. 2018; Griffin et al. 2019; Umoh et al. 2016), individuals at increased risk of CVD (n=1) (Vázquez-Fresno et al. 2015), individuals with overweight/obesity (n=2) (Meslier et al. 2020; Pagliai et al. 2020), individuals with chronic kidney disease (n=1) (Di Iorio et al. 2019), reactive hypoglycemia (n=1) (Quercia et al. 2017), non-frail and pre-frail participants (n=1) (Ghosh et al. 2020), individuals with and without mild cognitive impairment (n=1) (Nagpal et al. 2019), and individuals with coronary heart disease (CHD) from the CORDIOPREV study including sub-sets with and without metabolic syndrome (MetS; n=2) (Haro et al. 2016a; Santos-Marcos et al. 2019), with obesity (n=1) (Haro et al. 2016b), and with and without obesity and MetS (n=1) (Haro et al. 2017).

A summary of the MedDiet interventions are shown in Table 2. In four studies (Meslier et al. 2020; Nagpal et al. 2019; Quercia et al. 2017; Zhu et al. 2020), the participants were provided with MedDiet ingredients and/or meals, and in 8 studies participants were given adjunct counseling or access to a dietician (Djuric et al. 2018; Ghosh et al. 2020; Griffin et al. 2019; Haro et al. 2017, 2016b, 2016a; Santos-Marcos et al. 2019; Vázquez-Fresno et al. 2015). The full details of interventions of included studies are outlined in Supplementary Table 5.

### **Study quality and risk of bias**

Of the three prospective studies included in this systematic review, one study was deemed to have a moderate quality score of 6 stars (Pastori et al. 2017) and two studies a high score of  $\geq 7$  stars (Maskarinec et al. 2019; Wang et al. 2021) (Supplementary Table 1). Of the included cross-sectional studies, two studies had a low quality score of 0-3 stars (Cox et al. 2020; Pignanelli et al. 2018), six studies had a moderate score of 4-6 stars (Almanza-Aguilera et al., 2017; Filippis et al. 2016; Gallè et al. 2020; Garcia-Mantrana et al. 2018; Picchianti Diamanti et al. 2020; Ruiz-Saavedra et al. 2020) and six studies had a high quality score of  $\geq 7$  stars

(Barrea et al. 2019; Gutiérrez-Díaz et al., 2017; Gutiérrez-Díaz et al. 2016; Maldonado-Contreras et al. 2020; Mitsou et al. 2017; Valeriani et al. 2020) (Supplementary Table 2).

The quality of RCTs included in this systematic review was mixed (Figure 2, Supplementary Table 3). The risk of selection bias was considered to be low (43%) or unclear (57%; i.e., insufficient details provided to classify risk) across the identified studies. Performance bias was determined as low risk across all included studies, as blinding of participants and personnel to the nature of the intervention (which is difficult, if not impossible, in many dietary-pattern based interventions (Staudacher et al. 2017)) was deemed to have negligible effects on relevant outcome variables. The risk of detection and attrition bias was classified as low for  $\sim 50\%$  of included RCTs, and the remainder classified as unclear risk. Only 17% of included studies were classified as low risk in relation to reporting bias, with 28% classified as high risk, and for all remaining studies the risk was unclear. Other sources of bias were identified for four studies in relation to the management and analysis of data in which the MedDiet changes were reported relative to baseline rather than compared with the control (Djuric et al. 2018; Ghosh et al. 2020; Meslier et al. 2020; Rinott et al. 2021). The findings from the included studies are summarized in text below and in Supplementary Table 8, while individual study findings are described in more detail in Supplementary Text 1.

### **Findings from observational studies**

#### **Relationship between MedDiet and gut microbiota diversity**

Alpha-diversity metrics summarize the structure of the bacterial community with regards to its richness (i.e., number of taxonomic groups) and/or evenness (i.e., distribution of abundances of the groups), whereas Beta-diversity metrics summarize the degree to which samples differ from one another (Willis 2019). The association between MedDiet adherence and gut microbiota diversity was reported in 8 studies (Cox et al. 2020; Filippis et al. 2016; Gallè et al. 2020; Garcia-Mantrana et al. 2018; Maldonado-Contreras et al. 2020; Maskarinec et al. 2019; Picchianti Diamanti et al. 2020; Wang et al. 2021). Multiple different indexes were used across studies, with several investigations reporting effects of the MedDiet on more than one diversity index. A summary of the findings can be found in Figure 3A. Because different measures of alpha and beta diversity may yield different results, we have detailed the measures used in each study in Supplementary Table 6 and specific results summarized in Table 3.

Two (of seven) studies reported a relationship between MedDiet and alpha diversity assessed via the Shannon index. Cox et al. (2020) reported significantly higher Shannon index values in Turkish individuals with decompensated cirrhosis consuming a Med-style diet compared with a control group of American individuals consuming a Western diet. In a prospective study of healthy individuals from the USA, Maskarinec et al. (2019) observed a significant linear trend for higher Shannon index values with increasing



Table 2. Characteristics of randomized controlled trials investigating effects of the MedDiet on the gut microbiota.

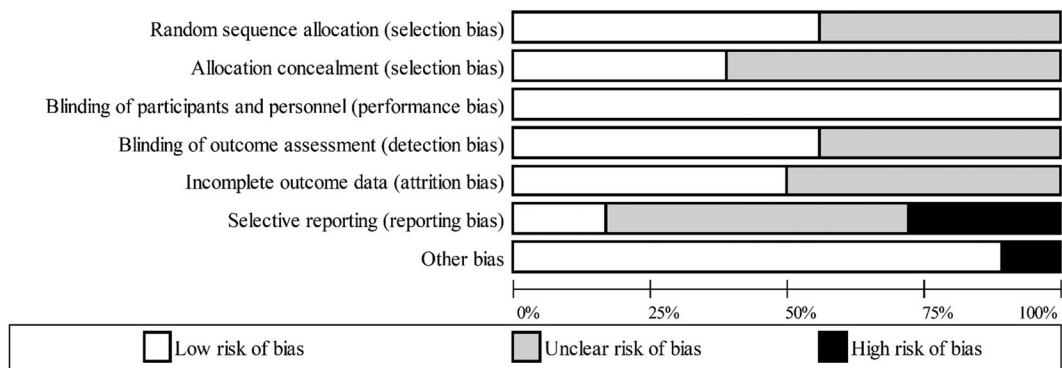
Authors	Country	Study design	Cohort	Health status	Sample size (n)	Male (n)	Age (y)	BMI (kg/m <sup>2</sup> )	Duration (weeks)	Type of Intervention	Type of control
Di Iorio et al. (2019)	Italy	Crossover	MEDIKA	Chronic kidney disease	60	49	68	27	26	MedDiet	Habitual diet
Djuric et al. (2018)	USA	Parallel	Healthy Eating for Colon Cancer Prevention	Increased risk of colon cancer	94	23	53	27	26	Modified MedDiet	Healthy eating diet
Ghosh et al. (2020)	Poland Italy France UK Netherlands	Parallel	NU-AGE	Non-frail and pre-frail	612	286	65-79	27	52	Modified MedDiet	Habitual diet plus national dietary guidelines leaflet
Griffin et al. (2019)	USA	Parallel	Healthy Eating for Colon Cancer Prevention	Increased risk of colon cancer	120	32	52	27	26	Med-style diet	Healthy eating diet
Haro et al. (2016a)	Spain	Parallel	CORDIOPREV	CHD without Metabolic syndrome	101	87	61	-	104	MedDiet	Low fat diet
Haro et al. (2016a)	Spain	Parallel	CORDIOPREV	CHD with Metabolic syndrome	138	111	60	-	104	MedDiet	Low fat diet
Haro et al. (2016b)	Spain	Parallel	CORDIOPREV	CHD with obesity	20	20	63	32	52	MedDiet	Low fat diet
Haro et al. (2017)	Spain	Parallel	CORDIOPREV	CHD with Metabolic syndrome and obesity	33	33	59	32	104	MedDiet	Low fat diet
Haro et al. (2017)	Spain	Parallel	CORDIOPREV	CHD without Metabolic syndrome but with obesity	32	32	64	33	104	MedDiet	Low fat diet
Haro et al. (2017)	Spain	Parallel	CORDIOPREV	CHD without Metabolic syndrome and without obesity	41	41	62	27	104	MedDiet	Low fat diet
Meslier et al. (2020)	Italy	Parallel	DINAMIC	Overweight/ obese	82	39	43	31	8	MedDiet	Habitual diet
Naggal et al. (2019)	USA	Crossover	BEAM	Mild cognitive impairment	17	5	65	NR	6	Modified Mediterranean ketogenic diet	American Heart Association Diet
Pagliari et al. (2020)	Italy	Crossover	CARDIVEG	Overweight	23	7	59	31	13	MedDiet	Vegetarian Diet
Park et al. (2019)	USA	Crossover	-	Healthy	14	-	31	23	4	South Beach Mediterranean diet	Atkins diet

(Continued)

Table 2. (Continued).

Authors	Country	Study design	Cohort	Health status	Sample size (n)	Male (n)	Age (y)	BMI (kg/m <sup>2</sup> )	Duration (weeks)	Type of Intervention	Type of control
Quercia et al. (2017)	Italy	Parallel	-	Reactive hypoglycemia	19	-	27-65	22-37	<1	Meddiet	Habitual diet
Rinott et al. (2021)	Israel	Parallel	DIRECT PLUS	Abdominally obese or dyslipidemic participants	90	82	52	32	26	MedDiet	Healthy dietary guidelines
Santos-Marcos et al. (2019)	Spain	Parallel	CORDIOPREV	CHD with Metabolic syndrome	79	79	62	31	156	MedDiet	Low fat diet
Santos-Marcos et al. (2019)	Spain	Parallel	CORDIOPREV	CHD with Metabolic syndrome	79	0	63	32	156	MedDiet	Low fat diet
Umoh et al. (2016)	USA	Parallel	Healthy Eating for Colon Cancer Prevention	Increased risk of colon cancer	120	34	53	27	26	Modified MedDiet	Healthy eating diet
Vázquez-Fresno et al. (2015)	Spain	Parallel	PREDIMED	High CVD risk	98	-	67	30	156	MedDiet+EVOO	Low fat diet
Zhu et al. (2020)	USA	Crossover	-	Healthy	10	5	22	24	<1	MedDiet+Nuts MedDiet	Fast food diet

CHD = coronary heart disease; CVD = cardiovascular disease; MedDiet = Mediterranean diet; EVOO = extra virgin olive oil;



**Figure 2.** Risk of bias for randomized controlled trials included in this systematic review.

MedDiet adherence. Other measures of alpha diversity that were reported in individual studies, including Chao1 (Garcia-Mantrana et al. 2018), Faith's Phylogenetic Diversity (Maldonado-Contreras et al. 2020), and an unspecified method (Filippis et al. 2016), were not significantly associated with the degree of MedDiet adherence.

In the two studies which measured beta-diversity via weighted and unweighted UniFrac distances, one reported no significant association with MedDiet score (Maldonado-Contreras et al. 2020), whilst the other (Maskarinec et al. 2019) reported significantly positively (unweighted axis 1 and weighted axis 2) or inversely (unweighted axis 2 and 3, weighted axis 1) associated weighted and unweighted UniFrac axes with MedDiet adherence level. Similarly, in the two studies measuring beta-diversity via Bray-Curtis dissimilarity, one reported no major differences in global structural variation of the gut microbiota (Wang et al. 2021), whilst the other reported significant dissimilarity in the bacterial profiles (Gallè et al. 2020).

### Relationship between MedDiet and bacterial abundance

Similar to microbiota diversity, deployment of different processing and analysis methods could give rise to difference in abundance measures and the methods used in the included studies are reported in [Supplementary Table 6](#). Overall 10 observational studies report the relationship between MedDiet and bacterial abundance at a minimum of one taxonomic level, as summarized in [Figure 3A](#) and [Table 3](#) (Gallè et al. 2020; Garcia-Mantrana et al. 2018; Gutiérrez-Díaz et al., 2017; Gutiérrez-Díaz et al. 2016; Maskarinec et al. 2019; Mitsou et al. 2017; Picchianti Diamanti et al. 2020; Ruiz-Saavedra et al. 2020; Valeriani et al. 2020; Wang et al. 2021). Half of the observational studies (3 of 6; [Supplementary Table 8](#)) reporting bacterial abundance at phyla level found a relationship with MedDiet. At lower taxonomic levels, relationships between MedDiet and bacterial abundance; (27 genera/species; [Table 3](#)) were observed mainly for *Faecalibacterium*, *Ruminococcus* (phylum Firmicutes) and *Bacteroides* (Phylum Bacteroidetes).

### Relationships between MedDiet adherence and gut microbial metabolites

Eleven studies reported relationships between MedDiet adherence and microbial metabolites in feces, urine or

plasma (Almanza-Aguilera et al., 2017; Barrea et al. 2019; Filippis et al. 2016; Garcia-Mantrana et al. 2018; Gutiérrez-Díaz et al., 2017; Gutiérrez-Díaz et al. 2016; Maldonado-Contreras et al. 2020; Mitsou et al. 2017; Pastori et al. 2017; Pignanelli et al. 2018; Ruiz-Saavedra et al. 2020).

Five studies reported associations between MedDiet adherence and the major SCFAs (Filippis et al. 2016; Gutiérrez-Díaz et al. 2016; Mitsou et al. 2017; Ruiz-Saavedra et al. 2020) (Maldonado-Contreras et al. 2020). Three out of 5 studies reported positive associations between MedDiet adherence and acetate (Filippis et al. 2016; Mitsou et al. 2017; Ruiz-Saavedra et al. 2020), propionate (Filippis et al. 2016; Gutiérrez-Díaz et al. 2016; Ruiz-Saavedra et al. 2020) and butyrate (Filippis et al. 2016; Gutiérrez-Díaz et al. 2016; Ruiz-Saavedra et al. 2020). Other microbial metabolites reported infrequently included other fatty acids, toxins and polyphenol metabolites ([Table 3](#)).

### Findings from randomised controlled trials

#### Effects of MedDiet on gut microbiota diversity

The effect of MedDiet on microbial diversity metrics was reported in 11 RCTs (Di Iorio et al. 2019; Djuric et al. 2018; Ghosh et al. 2020; Haro et al. 2017, 2016b; Meslier et al. 2020; Nagpal et al. 2019; Pagliai et al. 2020; Quercia et al. 2017; Rinott et al. 2021; Zhu et al. 2020). No studies found an effect of MedDiet on alpha- or beta-diversity when compared with the control group ([Figure 3B](#)). However, in a pooled analysis of data from all participants in one study, in which participants were classified by their level of adherence to a MedDiet during the intervention, a significant decline in diversity was observed in individuals with low and medium MedDiet adherence (at baseline), whereas high MedDiet adherence attenuated the loss of diversity with time (Ghosh et al. 2020).

#### Effects of mediterranean diet on bacterial abundances

Fourteen RCTs reported the effect of MedDiet intervention on bacterial abundance at different taxonomic levels (Di Iorio et al. 2019; Djuric et al. 2018; Ghosh et al. 2020; Haro et al. 2017, 2016a, 2016b; Meslier et al. 2020; Nagpal et al. 2019; Pagliai et al. 2020; Quercia et al. 2017, 2017; Rinott et al. 2021; Santos-Marcos et al. 2019; Zhu et al. 2020). In contrast with the observational studies, the majority of

Table 3. Results summary of observational studies investigating associations between MedDiet adherence and the gut microbiota.

Authors	Diversity	Taxonomic composition			Change in relative or absolute abundance (where reported) and direction of change	Microbial metabolites
		Phylum	Genus	Species		
Almanza-Aguilera et al. (2017)	N/A	N/A			Increase in TMAO	
Barrea et al. (2019)	N/A	N/A			Decrease in TMAO	
Cox et al. (2020)	Increase in Shannon index	N/A			N/A	
De Filippis et al. (2016)	No effect on alpha diversity (assessment method not specified)	N/A			High MedDiet associated with an increase in butyrate, acetate and propionate and reduced TMAO	
Picchianti Diamanti et al. (2020) <sup>a</sup>	No effect on Shannon index				N/A	
Galle et al. (2020)	No effect on Shannon index, significantly different Bray-Curtis distance.		<i>Coprobacillus</i>		↑	
			<i>Eubacterium</i>		↑	
			<i>Faecalibacterium</i>		↑	
			<i>Marseillibacter</i>		↑	
			<i>Tetragenococcus</i>		↑	
			<i>Dorea</i>		↑	
			<i>Methanobrevibacter</i>		↑	
			<i>Romboutsia</i>		↑	
			<i>Lachnospira Oscillospira</i>		↓	
			<i>Lactobacillus</i>	0.37	0.75	↓
	<i>Ruminococcus</i>	2.18	1.73	↓		
	<i>Lactococcus</i>	0.29	1.26	↓		
	<i>Veillonella</i>	9.51	7.63	↑		
	<i>Paraprevotella</i>	0.22	0.53	↑		
		0.09	0.14	↑		
		0.23	0.13	↓		
García-Mantrana et al. (2018)	No effect on Shannon index or Chao1.		<i>Blautia</i>		↓	Increase in total SCFAs
			<i>Streptococcus</i>		↓	
			<i>Canteniibacterium</i>		↑	
			<i>Clostridium</i>		↓	
			<i>Bacteroides</i>		↓	
Gutiérrez-Díaz et al. (2016)	N/A	↑Bacteroidetes ↓Firmicutes	<i>Prevotella</i>	0.29%	↑	Increase in propionate and butyrate, no effect on acetate.
			<i>R-Ruminococcus</i>	0.89%	↓	
Gutiérrez-Díaz et al. (2017)	N/A		<i>Clostridium cluster XIVa</i>	5.81	↑	Increase in benzoic acid, 3-hydroxyphenyl acetic acid. No effect on phenylacetic acid, phenylpropionic acid, 3-(3'-hydroxyphenyl) propionic acid, 4-hydroxyphenyl acetic acid, vanillic acid, syringic acid, phthalic acid or γ-valerolactone.
			<i>Faecalibacterium</i>	6.12	↑	

(Continued)

Table 3. (Continued).

Authors	Diversity	Phylum	Taxonomic composition		Change in relative or absolute abundance (where reported) and direction of change	Microbial metabolites
			Genus	Species		
Maldonado-Contreras et al. (2020) <sup>b</sup>	N/A	N/A				No effect on acetate, propionate and butyrate
Maskarinec et al. (2019)	Increase in Shannon index and both increase and decreases in weighted and unweighted UniFrac axes	↓Actinobacteria	<i>Lachnospiraceae incertae sedis</i>		↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑	N/A
Mitsou et al. (2017)	N/A	N/A	<i>Anaerostipes</i> <i>Coprococcus</i> <i>Lachnospira</i> <i>Faecalibacterium</i> <i>Ruminococcus</i> <i>Collinsella</i> <i>Escherichia</i> <i>Ruminococcaceae incertae sedis</i> <i>Acidaminococcus</i> <i>Bacteroides</i> <i>Escherichia</i>	<i>B. spp.</i> <i>E. coli</i>	↑ ↑ ↑	Increase in molar ratio of acetate and decrease in molar ratio of caproic acid with high vs low MedDiet. No difference in total SCFA, propionate, butyrate, iso-butyrate, iso-valerate, iso-caproic acid, valerate and heptanoic acid.
Pastori et al. (2017)	N/A	N/A				Decrease in serum lipopolysaccharides.
Pignagnelli et al. (2018)	N/A	No effect				No effect on TMAO, p-cresylsulfate, hippuric acid, indoxyl sulfate, p-cresyl glucuronide, phenyl acetyl glutamine or phenyl sulfate
Ruiz-Saavedra et al. (2020)	N/A		<i>Faecalibacterium</i> <i>Lactobacillus</i>		↑ ↓	Increase in acetate, propionate and butyrate. N/A
Valeriani et al. (2020)	N/A	No effect				N/A
Wang et al. (2021) <sup>e</sup>	No effect on Shannon index or Bray-Curtis dissimilarity		<i>Eubacterium</i> <i>Faecalibacterium</i> <i>Bacteroides</i> <i>Clostridium</i> <i>Collinsella</i> <i>Ruminococcus</i>	<i>E. eligens</i> <i>F. prausnitzii</i> <i>B. cellulosilyticus</i> <i>C. leptum</i> <i>C. aerofaciens</i> <i>R. torques</i>	↑ ↑ ↑ ↑ ↑ ↑	N/A

MedDiet = Mediterranean diet, SCFA = short chain fatty acids, TMAO = Trimethylamine N-oxide.

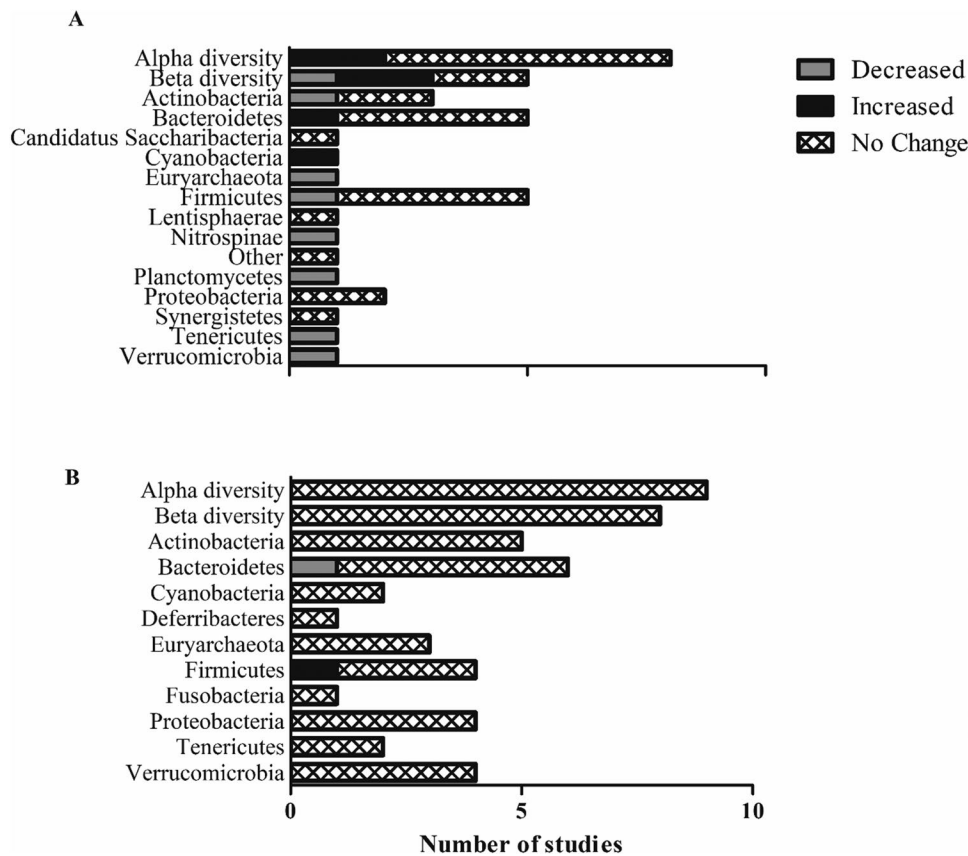
<sup>a</sup> Due to large number reported only associations between MedDiet adherence reported, abundance differences in Bacteria in High vs low/mod adherence in supplementary table

<sup>b</sup> Due to limited representation of persons with diets reflecting high conformance to MedDiet, examining differences in the gut microbiome between high and low adherence groups was not reported in this study.

<sup>c</sup>Culture based

<sup>d</sup>Data not reported in article

<sup>e</sup>Only those with q value < 0.1



**Figure 3.** Summary of findings for the effect of MedDiet on microbiota diversity and phylum abundance from (A) observational studies and (B) randomized controlled trials.

included RCTs found that MedDiet intervention had no effect on bacterial abundance at phyla level (Figure 3B). One of six studies found a significant effect at phylum level. Di Iorio and colleagues (2019) reported increased Firmicutes and decreased Bacteroidetes after MedDiet compared to control (habitual) diet in a crossover trial of individuals with chronic kidney disease. Across the included studies, MedDiet intervention was reported to have a significant effect on the abundance 37 genera of bacteria (direction of change in Table 4), but, overall, there appeared to be no consistent pattern of change. The exception was for a few studies which found that, at species level, *Collinsella aerofaciens* (Phylum Actinobacteria) was reduced whereas *Roseburia hominis* (Phylum Firmicutes) was increased with MedDiet. The most commonly investigated species was *Faecalibacterium prausnitzii* (Phylum Firmicutes) which was increased by MedDiet in 4 out of six studies.

#### Effects of MedDiet intervention on microbial metabolites

Ten studies report the effect of MedDiet on microbial metabolites (Di Iorio et al. 2019; Griffin et al. 2019; Meslier et al. 2020; Nagpal et al. 2019; Pagliai et al. 2020; Park et al. 2019; Quercia et al. 2017; Umoh et al. 2016; Vázquez-Fresno et al. 2015; Zhu et al. 2020; Table 4), with only four short-term interventions focusing on the main SCFA (Meslier et al. 2020; Nagpal et al. 2019; Pagliai et al. 2020; Quercia et al. 2017). There were no reported effects on fecal

concentrations of acetate following MedDiet intervention in any study (Meslier et al. 2020; Nagpal et al. 2019; Pagliai et al. 2020; Quercia et al. 2017). In contrast, one of the four studies reported higher fecal butyrate concentrations (Nagpal et al. 2019) and another an increase in propionate (Pagliai et al. 2020) after 6 and 13 weeks of MedDiet, respectively.

## Discussion

The current systematic review identified 17 RCTs and 17 observational studies which investigated the influence of MedDiet on the gut microbiota and microbial metabolites. Overall, the results suggest that there is little evidence that MedDiet affects microbiota diversity but some evidence that this dietary pattern may modulate taxonomic composition and SCFA production. However, the heterogeneity in both design and findings from the available studies precludes firm conclusions at this stage.

Investigation of the relationships between the MedDiet and the gut microbiome is hugely challenging. This is not only because of the inherent complexity of the MedDiet pattern, but also the diversity in a) methods used to assess adherence to this dietary pattern, b) the heterogeneity in the particular foods, and their relative amounts, that have been manipulated in the MedDiet interventions, c) whether participants were provided with supplementary ingredients/

Table 4. Results summary of randomized controlled trials investigating effects of the MedDiet on the gut microbiota.

Authors	Diversity	Taxonomic composition			Change in relative or absolute abundance (where reported) and direction of change	Microbial metabolites
		Phylum	Genus	Species		
Di Iorio et al. (2019)	No effect on alpha diversity (assessment method not specified).	↓Bacteroidetes	Bifidobacterium Collinsella Bacteroides	B. adolescentis C. aerofaciens B. coprocola B. thetaiotaomicron B. uniformis B. vulgatus P. merdae E. durans E. lactis E. cylindroides L. fermentum L. salivarius A. zavarzinii R. callidus R. gnavus S. anginosus S. sobrinus	1.22%	Decrease in indoxyl sulfate and p-cresyl sulfate. No effect on D-lactate.
					0.56%	
					1.41%	
					0.00%	
					0.04%	
					0.16%	
					0.32%	
					0.17%	
					0.09%	
					0.76%	
					0.00%	
					0.39%	
					0.26%	
					7.61%	
					0.18%	
0.08%						
1.00%						
0.67%						
		N/A				
Djuric et al. (2018)	No effect on Shannon index or Simpson's index or s θYC community distance index.	No effect			N/A	
Ghosh et al. (2020)a	No effect on Shannon diversity indices in individual countries (UK, France, Netherlands, Italy and Poland)	No effect	Faecalibacterium Roseburia Eubacterium  Bacteroides Prevotella Anaerostipes Ruminococcus Collinsella Coprococcus Dorea Clostridium Veillonella Flavonifractor Actinomyces	F. prausnitzii, R. hominis E. rectale E. eligens E. xylanophilum B. thetaiotaomicron, P. copri A. hadrus R. torques C. aerofaciens C. comes D. formicigenans C. ramosum V. dispar F. plautii A. lingnae	↑	
					↑	
					↑	
					↑	
					↑	
					↑	
					↑	
					↑	
					↑	
					↑	
					↑	
					↑	
					↑	
					↑	
Griffin et al. (2019)	N/A	N/A			No effect on TMAO	
Haro et al. (2016a) - CHD without metabolic syndrome	N/A	E. rectale	Eubacterium	E. rectale	1.08	N/A
					1.90	
Haro et al. (2016a) - CHD with metabolic syndrome	N/A	Parabacteroides Bacteroides Faecalibacterium Bifidobacterium	P. distasonis B. thetaiotaomicron F. prausnitzii B. adolescentis B. longum	P. distasonis B. thetaiotaomicron F. prausnitzii B. adolescentis B. longum	1.79	N/A
					1.29	
					1.78	
					1.40	
					1.58	
		N/A				

(Continued)





Table 4. (Continued).

Authors	Diversity	Taxonomic composition			Change in relative or absolute abundance (where reported) and direction of change			Microbial metabolites
		Phylum	Genus	Species				
Nagpal et al. (2019)	No effect on unmer observed OTUs, phylogenetic diversity, Chao1, Shannon index or weighted and unweighted UniFrac distance.		<i>Bifidobacterium</i>					Butyrate was increased by MedDiet but there was no effect on propionate, acetate or D-lactate.
Pagliai et al. (2020)	No effect on Richness, Simpson's index, Gini-Simpson's index, inverse Simpson index, Shannon index, Evenness, Dominance, weighted and unweighted UniFrac distance or Bray-Curtis dissimilarity		<i>Anaerostipes</i> <i>Clostridium sensu stricto</i> <i>Enterorhabdus</i> <i>Veillonella</i>		58% -18% -17% -1%	↓ ↑ ↑ ↑		MedDiet increased propionate but had no effect on butyrate, acetate, isobutyrate, isovalerate or valerate.
Park et al. (2019)	N/A		N/A					No effect on TMAO.
Quercia et al. (2017) <sup>d</sup>	No effect on Bray-Curtis dissimilarity.		<i>Coproccoccus</i> <i>Enterococcus</i> <i>Anaerofustis</i> <i>Dorea</i> <i>Bacteroides</i> <i>Campylobacter</i> <i>Brenneria</i> <i>Veillonella</i> <i>Akkermansia</i> <i>Bacteroides</i>					No effect on acetate, butyrate or propionate
Rinott et al. (2021) <sup>b</sup>	No effect on weighted UniFrac distance.		<i>A. muciniphila</i> <i>B. caccae</i> <i>B. darus</i> <i>R. hominis</i> <i>R. lactaris</i> <i>Sp. Marseille P3087</i> <i>C. comes</i> <i>B. angulatum</i> <i>B. cantenulatum</i> <i>B. longum</i> <i>B. bifidum</i> <i>B. adolescentis</i> <i>L. ruminis</i>			→ → → → → → → → → → → →		N/A
Santos-Marcos et al. (2019) - males <sup>b</sup>	N/A		<i>Lactobacillus</i> <i>Roseburia</i> <i>Holdemania</i>					N/A
Santos-Marcos et al. (2019) - females <sup>b</sup>	N/A		<i>Desulfovibrio</i>			↑		N/A
Umoh et al. (2016)	N/A		N/A					No change in total BCFA (Continued)

Table 4. (Continued).

Authors	Diversity	Taxonomic composition			Change in relative or absolute abundance (where reported) and direction of change	Microbial metabolites
		Phylum	Genus	Species		
Vázquez-Fresno et al. (2015)	N/A		N/A			Decreased TMAO with MedDiet + extra virgin olive oil, no effect of TMAO with MedDiet + nuts. Increase in P-cresol and isobutyrate with MedDiet + nuts and MedDiet + extra virgin olive oil.
Zhu et al. (2020)	No effect on Alpha diversity (method not specified) or weighted UniFrac distances		<i>Collinsella</i> <i>Butyrivibrio</i>	1.62% 0.07%	↓ ↑	No effect on TMAO, deoxycholic acid, lithocholic acid, urodeoxycholic acid, taurodeoxycholic acid, glycodeoxycholic acid, glycooursodeoxycholic acid, glycohyodeoxycholic acid An increase in hippuric acid.

MedDiet = Mediterranean diet, TMAO = Trimethylamine N-oxide.

<sup>a</sup>Enriched (↑) or depleted (↓) with increased MedDiet adherence.

<sup>b</sup>Change compared to baseline

<sup>c</sup>Data does not include unclassified species; enriched with MedDiet (↑) or control diet (↓).

<sup>d</sup>Dissimilar Multidimensional scaling from based on Bray-Curtis baseline MedDiet.

meals or simply recommendations to change their diet, and d) the duration of intervention (Hutchins-Wiese, Bales, and Porter Starr 2021). In addition, the human gut microbiome is a complex ecosystem including bacteria, archaea and fungi plus a vast array of viruses, most of which are bacteriophages that infect, and replicate in, bacteria and archaea. Although it is generally accepted that diet is a major factor influencing the gut microbiome, there is very limited understanding of causal relationships between what is eaten and the gut microbiota in humans (Leeming et al. 2021). The issue is compounded by lack of understanding of, and limited consensus on, the characteristics of a healthy microbiome (Lloyd-Price, Abu-Ali, and Huttenhower 2016; McBurney et al. 2019) so that differences in the microbiome associated with different diets, or dietary patterns, are difficult to interpret. While a number of studies have suggested associations between specific bacteria and some disease states, these are often disease-specific and the current literature is conflicting (Chen et al. 2021; Palmu et al. 2020). On the other hand, high microbiota diversity appears to be a consistent feature of a healthy gut microbiota (Lloyd-Price, Abu-Ali, and Huttenhower 2016), and loss of diversity has been associated with increased disease risk (He et al. 2018; Lloyd-Price, Abu-Ali, and Huttenhower 2016). Overall, there was no conclusive evidence that MedDiet affects gut microbiota diversity. However, the absence of conclusive evidence may reflect limitations in the studies to date and, in particular, may be due to the relatively small size of most of the prospective cohort studies included in this analysis. For example, in two prospective studies of healthy adults in the USA, only the larger study ( $n=1735$  vs  $307$ ) found an association between MedDiet and alpha-diversity (Maskarinec et al. 2019; Wang et al. 2021).

Furthermore, health status is likely an important factor in determining effects of the MedDiet on the gut microbiota. For example, Cox et al. (2020) found a higher Shannon index in Turkish individuals with decompensated cirrhosis consuming a Med-style diet compared with a control group of American individuals consuming a Western diet, but not healthy controls or those with compensated cirrhosis. This is further supported by the CORDIOPREV RCT in which two years of MedDiet increased *Bacteroides*, *Prevotella*, *Roseburia* and *Ruminococcus* in males with MetS and obesity but not in obese males without MetS or non-obese males without MetS (Haro et al. 2017). These genera were also reduced in the participants with MetS and obesity compared with the non-obese individuals without MetS, suggesting that MedDiet may have potential to restore eubiosis, but that this is dependent on baseline abundance of bacteria. Indeed, individuals living with obesity and different disease states have different enterotypes, and may respond differently to dietary interventions (Arumugam et al. 2011). However, it is important to highlight that observed effects could also be confounded by medication use. Further research is needed to determine how different health conditions, and the medications used to treat those conditions, modulate the effects of diet on the gut microbiota.

Other characteristics of the human cohorts including age, sex, geography and ethnicity, as well as study durations may contribute to the heterogeneity in findings and make it difficult to draw clear and well-evidenced conclusions. In an attempt to identify potential differences between shorter and longer duration studies, we compared the effects of short-term and medium-long term MedDiet interventions on the gut microbiota (Supplementary Table 8). However, this did not reveal any clear differences associated with study duration. This finding is likely due to the considerable heterogeneity (as outlined above) in other methodological aspects between studies. As an example of the complexity in comparing the findings between different studies, Gutiérrez-Díaz et al. (2016) found that higher adherence to MedDiet was associated with higher Bacteroidetes and lower Firmicutes in a study of healthy middle-aged Italians. However, the same authors report no such relationship in cohort of healthy older Spanish people (Gutierrez-Diaz et al., 2017). Both studies were cross-sectional and the authors used the same MedDiet scoring tool, which suggests that study design and the way in which the MedDiet was characterized are unlikely to explain key differences between the two investigations. The difference in finding could instead relate to the different age ranges in the two studies (Mariat et al. 2009), differences in the study setting, and other exposure or phenotypic differences between the two cohorts.

In light of the challenges described above, it is unsurprising that very few studies found an effect of MedDiet on bacterial abundance at phylum level, and none of the studies reported significant effects on Firmicutes/Bacteroidetes ratio. MedDiet appeared to affect bacterial composition at lower taxonomic levels but such effects were not consistent. There was some evidence, mainly from RCTs, that MedDiet reduced bacterial species that preferentially utilize oligosaccharides and simple sugars (e.g. *C. aerofaciens* and *B. adolescentis*) and increased those with an affinity for polysaccharides (e.g. *R. hominis*, *B. thetaiotaomicron* and *E. eligans*) (Clavel et al. 2016; De Angelis et al. 2015). Additionally, two studies found that higher MedDiet adherence was associated with greater abundance of *A. equolifaciens* which is involved in polyphenol metabolism (Picchianti Diamanti et al. 2020; Wang et al. 2021). Notably, both observational studies and RCTs found that MedDiet may increase *F. prausnitzii*, a keystone butyrate-producer associated with anti-inflammatory activities (Velasquez-Manoff 2015). However, MedDiet appeared to affect different *F. prausnitzii* strains in different studies (Ghosh et al. 2020; Meslier et al. 2020) which could have implications for SCFA metabolism and any consequential anti-inflammatory effects (Martín et al. 2017). Moreover, as is common in the complex gut microbial ecosystem, *F. prausnitzii* have been shown to cross-feed and to cooperate with, and to compete with, other bacterial species (Flint et al. 2007; Lindstad et al. 2021). A more MedDiet-like eating pattern will produce complex changes in the substrates flowing to the large bowel, each of which may have positive or negative effects on multiple members of this complex microbial ecosystem.

Further variability is introduced by the different components and quantities of foods in the MedDiet administered (especially the quantity of prebiotics such as fiber and polyphenols (Gibson et al. 2017; Tuohy et al. 2012)). As a consequence, it may be naive to assume that the MedDiet would provoke simple, characteristic changes in the microbiota at any taxonomic level. It has been proposed that a more holistic approach that goes beyond characterization of bacterial composition and encompasses dynamic interactions between all components of the microbiota may help in investigation of links with health of the host (Clemente et al. 2012) and the same is likely to apply when attempting to understand changes in response to dietary patterns such as MedDiet. On that basis, dietary manipulation of the microbial ecosystem would be expected to result in complex (and, perhaps, difficult to predict) changes in the patterns of metabolites that are end-products of the multiple biochemical interactions between microbes and that can be measured in stool. Nevertheless, it is also likely that the pattern of these microbial metabolites (e.g. SCFA) will be critical in determining consequences for host physiology (Leeming et al. 2021). Despite this, few studies reported the effects of MedDiet on microbial metabolites, and we suggest that this should be a priority for future research.

### **Strengths and limitations**

This is the first systematic review to explore effects of MedDiet on the composition and metabolism of the gut microbiota. A strength of this review is the inclusion of a large number of both RCTs and observational studies, which provide complementary insights into the impact of MedDiet on the gut microbiota. For example, inclusion of observational studies allowed us to explore the impact of the MedDiet in a ‘real-world’ setting, where cause-effect relationships cannot be established, but where larger sample sizes and longer follow ups are available compared with RCTs. This is an important factor, given that shifts in the microbiota change rapidly in response to dietary interventions (David et al. 2014) but whether such changes are sustained in the longer-term effects is unclear. Inclusion of RCTs allowed us to assess the evidence for causal effects of the MedDiet on gut microbiota in more controlled experimental settings, where the characteristics of the intervention may be well defined (albeit that the nature and duration of such interventions varied between studies). Several narrative reviews have explored the effects of the MedDiet on the gut microbiota (Del Chierico et al. 2014; García-Montero et al. 2021; Merra et al. 2020; Tsigalou et al. 2021). These usually include a small number of studies and report mainly positive findings and there is a significant inherent risk of selection bias in narrative reviews. Our systematic review includes data on gut microbiota metabolites, especially SCFAs, which are likely to be pivotal in mediating effects on human health (van der Hee and Wells 2021). Further strengths of this review include adherence to the PRISMA guidelines and prospectively registering our review protocol, which minimizes reviewer bias. In

addition, our searches were conducted by an information specialist who is experienced with systematic review methodologies and used multiple databases whilst including gray literature to maximize our chance of identifying all relevant research. Nevertheless, despite our robust methods, it is possible that we did not identify all relevant studies in this area. A limitation of the evidence included in this systematic review was the mixed quality and heterogeneity of included studies, including some issues surrounding data analysis, which stresses the need for further well-controlled investigations in this area. A further limitation is that we were unable to quantitatively synthesize results from different studies via meta-analysis, because of substantial diversity in outcome measurements and the limited number of studies exploring effects of the MedDiet on consistent outcomes.

### **Conclusions and directions for future research**

Although there is some evidence from a small number of studies indicated a positive impact of a MedDiet on specific gut microbiota, the findings of this systematic review suggest that this dietary pattern does not consistently alter microbiota composition or metabolism. This lack of a consistent effect is not surprising given the heterogeneity between study populations, analysis methods, duration and characterization of MedDiet and the limited approaches that have been used to characterize the complex ecosystem that constitutes the large bowel microbiome. This research area will benefit from improved conceptualization of, and compelling evidence for, the characteristics of a “healthy gut microbiome” (Goodrich et al. 2014; McBurney et al. 2019) that could be used as an external reference against which to judge the effects of any changes in the gut microbiome associated with the MedDiet or other dietary patterns/interventions. Further, given the differential effects of individual components of the MedDiet on the gut microbiota, this research area will also benefit from standardization of the composition of experimental MedDiet interventions and from standardization of the scoring methods used to assess MedDiet adherence in observational studies. Finally, the development, and adoption, of open access systems for data sharing would facilitate meta-analyses at individual participant level that may help to resolve some of the heterogeneity in reported findings.

### **Disclosure statement**

No potential conflict of interest was reported by the authors.

### **Author contributions**

This study was conceived by OMS and JCM. The study was planned and designed by RK, PG, AA, CS, KD, JM, AG, FCM, AJ, DH, ES, AMM, MS, OMS and JCM. JM designed the search strategy and performed the searches. RK and PG screened the titles, abstracts, and full-texts of retrieved papers, with any disputes resolved in consultation with OMS. RK and PG extracted data. AG conducted the risk

of bias assessment, which was verified by OMS. RK, CS, FCM, AJ, DH, OMS and JCM interpreted the data. OMS and RK drafted the manuscript. PG, AA, CS, KD, JM, AG, FCM, AJ, DH, ES, AMM and MS further contributed toward the writing and critical revision of the manuscript. All authors have read and approved the final version of the manuscript.

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