

Effects of the Mediterranean Diet on Gut Microbiota and Cardiometabolic Outcomes in Adults with Overweight or Obesity: A Systematic Review of Randomized Controlled Trials (RCT)

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ABSTRACT

Obesity is associated with metabolic dysfunction and alterations in the gut microbiota. The Mediterranean diet (MD), rich in plant-based foods, dietary fiber, polyphenols, and unsaturated fats, may influence microbial composition and cardiometabolic health. This systematic review evaluated the effects of MD interventions on gut microbiota composition, microbial functions, and cardiometabolic outcomes in adults with overweight or obesity. The review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines and registered in PROSPERO (CRD420251155212). Randomized controlled trials examining MD interventions in adults with overweight or obesity were eligible. We used the Cochrane Risk of Bias 2 (RoB 2) tool to assess the risk of bias and the GRADE approach to evaluate the certainty of evidence. Five publications representing three independent cohorts were included. A narrative synthesis was performed due to overlapping study populations, methodological heterogeneity, and variability in microbiome assessment methods. MD interventions were associated with changes in specific gut microbial taxa, including short-chain fatty acid-producing bacteria such as *Faecalibacterium*, *Roseburia*, and *Butyricicoccus*. Additional studies reported changes in microbial diversity and cardiometabolic markers. However, the certainty of evidence was rated low to very low due to study design limitations, small sample sizes, heterogeneity, and potential risk of bias. In conclusion, MD interventions may influence selected gut microbial taxa and cardiometabolic outcomes in adults with overweight or obesity, though further well-designed randomized controlled trials are required to establish clinical relevance.

INTRODUCTION

Obesity represents a major global health challenge and is associated with an increased risk of cardiometabolic disorders, including cardiovascular disease, metabolic syndrome, and type 2 diabetes. Beyond excessive adipose tissue accumulation, obesity is characterized by complex metabolic disturbances involving chronic low-grade inflammation and alterations in

gut microbiota composition.¹ The gut microbiota contributes to host metabolic regulation, immune homeostasis, energy balance, and inflammatory processes; therefore, disruption of microbial composition may influence obesity-related metabolic dysfunction.^{2,3}

Dietary patterns have been increasingly investigated as potential modulators of obesity-related metabolic alterations.⁴ These dietary approaches include a high content of plant-based foods, dietary fiber, polyphenols, monounsaturated fatty acids, whole grains, nuts, fish and limited consumption of red and processed meats.^{5,6} Previous studies have linked higher adherence to the Mediterranean Diet with better cardiometabolic profiles, including improved glycemic control, lower levels of inflammatory markers and better lipid metabolism.⁷

The potential metabolic effects of Mediterranean Diet have been partly attributed to its interaction with gut microbiota.⁸ Components of the Mediterranean Diet, in particular fiber and polyphenol-rich foods, may affect the composition of microbes and favor the growth of bacteria involved with short-chain fatty acid (SCFA) production.⁹ Taxa such as *Faecalibacterium prausnitzii*, *Roseburia*, *Lachnospiraceae* and other fiber-fermenting microorganisms may influence microbial fermentation and host metabolic regulation.¹⁰

However, the effects of Mediterranean Diet interventions on gut microbiota composition are still heterogeneous.¹¹ Variability between studies may be attributed to differences in dietary intervention protocols, participant characteristics, adherence rates, duration of intervention, microbiome sequencing techniques and analytical methods.¹² Furthermore, whether the microbiome changes associated with Mediterranean Diet contribute to clinically relevant metabolic and inflammatory outcomes in people with obesity remains unknown.¹³

Hence, a systematic evaluation of randomized controlled trials is required to clarify the current evidence about the relationship between Mediterranean Diet interventions, alterations in gut microbiota and metabolic outcomes in obese subjects. This systematic review aimed to assess the effects of Mediterranean Diet adherence on gut microbiota composition and related metabolic and inflammatory outcomes among individuals with obesity.

METHODS

Study Design

This systematic review was conducted according to the methodological recommendations of the Cochrane Handbook for Systematic Reviews of Interventions, Second Edition, and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement.¹⁴ The PICO (Population, Intervention, Comparison, and Outcomes) framework was applied to formulate the research question, define the eligibility criteria, and guide the systematic review methodology. The

review protocol was prospectively registered in the International Prospective Register of Systematic Reviews (PROSPERO) under registration number CRD420251155212 prior to conducting the literature search. The systematic review followed a predefined protocol consisting of study identification, screening, eligibility assessment, data extraction, risk of bias assessment, and evidence synthesis.

The review process was performed by independent reviewers to ensure methodological transparency and minimize possible bias. The procedures included systematic selection of studies, extraction of relevant study characteristics and outcomes, assessment of methodological quality, and synthesis of findings from eligible randomized controlled trials. Differences of opinion were resolved by discussion between reviewers. The reporting of this review followed PRISMA 2020 recommendations, including transparent documentation of the study selection process, characteristics of included studies, methodological assessment, and synthesis of evidence.

Table 1. Eligibility criteria for this study based on PICO framework

Domain	Inclusion Criteria	Exclusion Criteria
Population	<ul style="list-style-type: none"> Adults ≥ 18 years Overweight or obese individuals Both males and females Obesity criteria based on BMI for each population 	<ul style="list-style-type: none"> Pregnant or lactating women Children or adolescents (<18 years) Patients with acute illness or severe infection
Intervention	Mediterranean Diet (MD) in any form:	Dietary patterns that are not Mediterranean Diet
Comparison	Any non-Mediterranean control diet	-
Outcomes (Primary)	<ul style="list-style-type: none"> Changes in gut microbiota composition calculate by: 16S rRNA sequencing Shotgun metagenomics Gene richness / alpha–beta diversity Changes in specific taxa 	Studies that do not measure gut microbiota
Outcomes (Secondary)	Metabolomic changes (SCFAs, bile acids, carnitine, urolithins)	-
Study Design	Randomised Controlled Trials	Non RCT
Language & Publication	Full-text articles available in English	<ul style="list-style-type: none"> Abstract-only publications without full text Articles published in non-English languages

Search Strategy

A comprehensive literature search was conducted in PubMed and Scopus databases from inception to September 2025. The search strategy was developed using three main concept blocks: (1) population/problem related to obesity and overweight, (2) intervention related to the Mediterranean Diet, and (3) outcomes related to gut microbiota and microbial metabolites. Medical Subject Headings (MeSH) terms and free-text keywords were combined

using Boolean operators. The search strategy included terms related to obesity (“Obesity” [MeSH], obesity, overweight, adiposity), Mediterranean Diet (“Mediterranean Diet” [MeSH], Mediterranean diet, Mediterranean dietary pattern, MedDiet), and gut microbiota outcomes (“Gastrointestinal Microbiome” [MeSH], gut microbiota, gut microbiome, microbiome, microbial composition, microbial diversity, short-chain fatty acids, SCFA, metabolome, metabolomics, microbial metabolites). The search strategy was adapted for each database according to database-specific indexing systems and syntax. Studies were limited to human randomized controlled trials published in English. No restriction was applied regarding publication year, while the final search was conducted in September 2025.

Selection Criteria

Every obtained document per search from the database was transferred into Endnote. This included those RCTs which focused on Mediterranean dietary practices and gut microbiota in the obese populations. Three reviewers worked independently to examine the titles and then the abstracts of all records before moving on to a full text review for eligible studies. Any disagreements were settled through discussion and if no consensus was reached a third reviewer was appointed as an arbitrator. The selection of studies data was captured using the PRISMA 2020 flow diagram presenting the studies captured in the search, the studies to were screened, the studies excluded, and the studies included (with clear exclusion reasons).

Data Extraction

Data extraction was performed independently by two reviewers (MA and CLA) using a standardized data extraction form developed specifically for this systematic review. The extraction form was reviewed and piloted prior to the data extraction process to ensure consistency and completeness of the collected information. The extracted data included study characteristics (first author, publication year, country, and study design), participant characteristics (sample size, age, sex, and baseline health status), intervention characteristics (Mediterranean Diet protocol, duration, and adherence assessment), comparator characteristics, microbiome assessment methods, and reported outcomes related to gut microbiota composition and metabolic parameters. For microbiome-related outcomes, data extraction focused on reported changes in microbial diversity, taxonomic composition, and specific bacterial taxa associated with the Mediterranean Diet intervention. Additional reported outcomes, including metabolic markers and inflammatory parameters, were extracted when available and relevant to the review objectives.

When multiple publications reported findings from the same study population, these reports were assessed and linked to avoid double-counting participants. The findings were

considered as originating from a single cohort, and the most comprehensive report was used as the primary source while additional publications were used to provide complementary information. Any disagreements during the data extraction process were resolved through discussion between reviewers until consensus was reached.

Risk of Bias Assessment

The risk of bias of the included randomized controlled trials was assessed using the Cochrane Risk of Bias 2 (RoB 2) tool, as described in the *Cochrane Handbook for Systematic Reviews of Interventions*. The assessment was conducted across five domains: bias arising from the randomization process, bias due to deviations from intended interventions, bias due to missing outcome data, bias in measurement of the outcome, and bias in selection of the reported result. Two reviewers independently performed the risk of bias assessment for each included study.¹⁵ Each domain was classified according to the standard RoB 2 judgement categories: low risk of bias, some concerns, or high risk of bias. Disagreements between reviewers were resolved by discussion or consensus, with the involvement of a third reviewer when needed.

We assessed the certainty of the evidence for each outcome using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach.¹⁶ The certainty assessment was performed in five domains: risk of bias, inconsistency, indirectness, imprecision and publication bias. The overall certainty of evidence was rated as high, moderate, low or very low. A Summary of Findings table was developed to present the certainty of evidence for the main outcomes including gut microbiota composition, SCFA-related microbial pathways, *Akkermansia muciniphila* abundance and cardiometabolic outcomes. The certainty of evidence was assessed using the GRADE domains of risk of bias, inconsistency, indirectness, imprecision, and publication bias.

Level of Evidence

The evidence levels for the outcome measures were assessed using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) criteria and categorized into 4 levels as follows: high, moderate, low, and very low.¹⁷ The basis for this research hinges on a number of questions. Namely: Why has this line of research been unexplored? Why is GRADE reverse correlation? What is the most suitable design for this research? GRADE has five key domains: risk of bias, inconsistency, imprecision, indirectness, publication bias. Each of these is meant to assess other primary factors in addition to mere statistically significant results, as well as other factors contributing to validity and resultant certainty. To what degree will the opposing evidence be able to advance in the other core

domains of GRADE which are clarity, control and evidence synthesis? Therefore, these are the reasons this research must be a systematic review to use GRADE.¹⁴

Reporting and Ethical Consideration

This academic work conforms to the 2020 PRISMA standards. This protocol has been registered with PROSPERO (CRD420251155212) as this systematic review draws on secondary data and therefore, did not require ethics approval or consent from the participants.

RESULTS

Study Selection

A total of 404 records were identified through database searches, including 114 records from PubMed and 290 records from Scopus. After removing 103 duplicate records, 301 records underwent title and abstract screening. During this stage, records that were not related to the research question, not original research articles, or not involving the target population or intervention were excluded. A total of 286 records were sought for retrieval, of which five records could not be obtained. The remaining 281 full-text articles were assessed for eligibility. After full-text assessment, 276 articles were excluded due to reasons including irrelevant population ($n = 10$), inappropriate intervention ($n = 8$), absence of original data ($n = 228$), and irrelevant outcomes ($n = 30$). Finally, five publications reporting randomized controlled trials were included in this systematic review. The study selection process is presented in the PRISMA 2020 flow diagram (Figure 1).

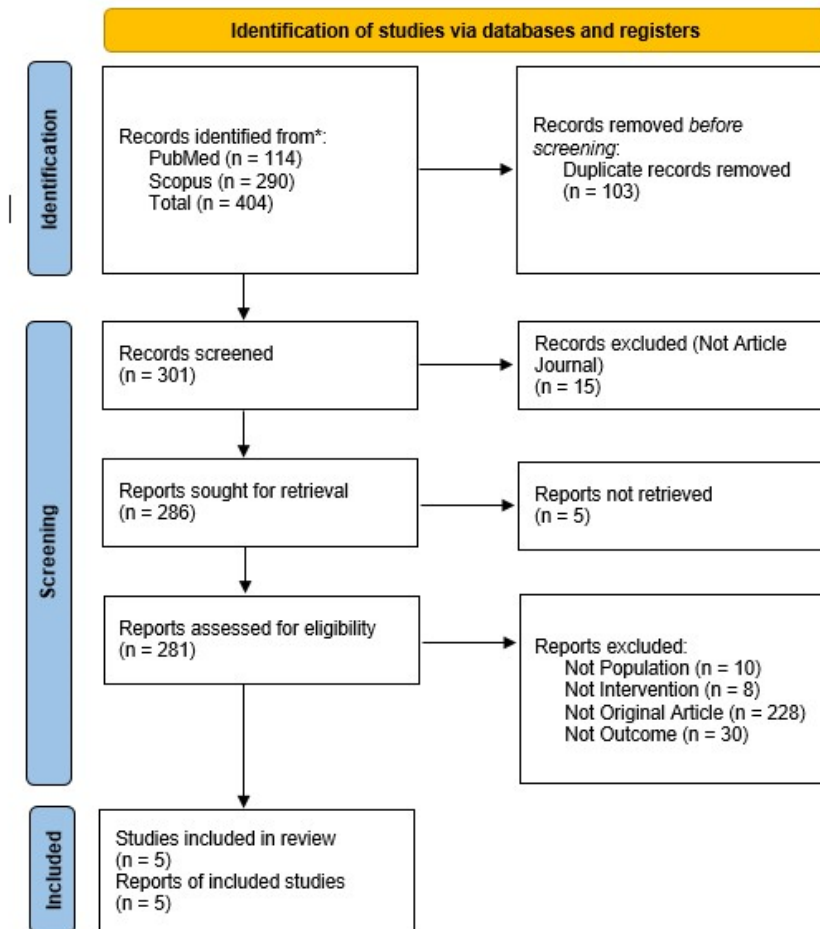


Figure 1. Prisma flow chart

Characteristics of Studies

Five publications describing randomized controlled dietary interventions met the eligibility criteria and were included in this systematic review (Table 2). However, these publications did not represent five completely independent study populations. Specifically, Meslier et al. and Tagliamonte et al. reported findings from the same cohort of 82 overweight or obese participants, while Haro et al. (2016) and Haro et al. (2017) represented different analyses from the CORDIOPREV study population.^{18,19} Therefore, the evidence synthesized in this review represents five study reports derived from approximately three independent cohorts.

The studies included in this review explored the effects of Mediterranean Diet patterns, Green-Mediterranean Diet patterns, and Mediterranean Diet supplemented with dairy products (MedDairy) -based interventions, on gut microbiota composition and associated metabolic outcomes. Dietary interventions ranged from 8 weeks to 2 years. Comparators were regular dietary patterns or low-fat dietary interventions. Included studies included adult

participants with overweight, obesity, cardiovascular risk factors or metabolic abnormalities. Gut microbiota changes were analyzed using different analytical approaches, including 16S rRNA sequencing, shotgun genome sequencing, and quantitative PCR-based microbial quantification. The outcomes included alterations in microbial diversity, the relative abundance of particular bacterial taxa, microbial functional pathways, and associations with cardiometabolic markers.

Meslier et al. evaluated an 8-week Mediterranean Diet intervention in 82 overweight or obese participants, reporting modifications in gut microbial composition, microbial gene richness and metabolic markers (lipid-related parameters and inflammatory markers).²⁰ A further analysis of the same cohort was performed by Tagliamonte et al. exploring associations between Mediterranean Diet adherence, changes in gut microbiota and endocannabinoid-related metabolic markers.²¹ Among their findings was an increased abundance of specific microbial taxa, including *Akkermansia muciniphila* after the intervention.

Choo et al. assessed a Mediterranean Diet supplemented with dairy products (MedDairy) in adults at cardiovascular risk using a randomized crossover design.²² The intervention did not result in significant changes in overall gut microbial community structure but was associated with changes in selected bacterial taxa, including increased abundance of *Butyricoccus*. Findings from the CORDIOPREV cohort were reported by Haro et al. The 2016 analysis focused on microbial changes in response to dietary intervention in patients with coronary heart disease, and the 2017 analysis examined longer-term metabolic and microbiome-related changes across metabolic phenotypes.¹⁸ Both studies reported changes in bacterial taxa associated with carbohydrate fermentation, short chain fatty acid production and metabolic health. There was significant methodological heterogeneity among included studies in intervention duration, participant characteristics, dietary comparison groups, microbiome sequencing techniques, and outcomes reported. These variations limited direct comparison between studies and were considered during the interpretation of findings.

Table 2. Characteristics of included studies evaluating the effects of Mediterranean diet interventions on gut microbiota

Author, year	Country	Study design	Study population and baseline characteristics	Intervention	Methods for measuring dietary adherence	Gut microbiota analysis
Haro, 2016	Spain	Prospective, randomized, open-label, controlled clinical trial, sub study of CORDIOPREV comparison: Low-Fat, High-Complex Carbohydrate Diet	20 male obesity <ul style="list-style-type: none"> • Age: 65.2 ± 3.2 years • Weight: 88.3 ± 1.8 kg • Waist Circumference: 109.6 ± 2.2 cm • BMI: 32.8 ± 0.5 kg/m² • OOGT: 91.2 ± 2.7 mg/dL • HbA1c: 6.0 ± 0.1 % • Systolic (SBP): 136.0 ± 3.7 mmHg • Diastolic (DBP): 75.1 ± 3.5 mmHg • Triglyceride (TG): 98.7 ± 7.6 mg/dL • Total Cholesterol (TC): 150.2 ± 7.0 mg/dL • HDL: 42.1 ± 1.9 mg/dL • LDL: 88.0 ± 6.4 mg/dL • Insulin 3.13 ± 0.58 	Med Diet <ul style="list-style-type: none"> • Weight: 88.3 ± 1.8 kg • Waist Circumference: 109.6 ± 2.2 cm • BMI: 32.8 ± 0.5 kg/m² • OOGT: 96.8 ± 3.5 mg/dL • HbA1c: 6.03 ± 0.13 % • Systolic (SBP): 136.0 ± 3.7 mmHg • Diastolic (DBP): 75.1 ± 3.5 mmHg • Triglyceride (TG): 85.8 ± 11.5 mg/dL • Total Cholesterol (TC): 148.2 ± 5.8 mg/dL • HDL: 44.8 ± 2.0 mg/dL • LDL: 85.9 ± 5.1 mg/dL • Insulin 6.02 ± 1.13 Genus <ol style="list-style-type: none"> 1. <i>Roseburia</i> <ul style="list-style-type: none"> • Increase • <i>P-value</i>: 0.017 2. <i>Oscillospira</i> <ul style="list-style-type: none"> • Increase • <i>P-value</i>: 0.016 3. <i>Prevotella</i> <ul style="list-style-type: none"> • Increase • <i>P-value</i>: 0.028 	<ul style="list-style-type: none"> • Mediterranean Diet Adherence Score (MEDAS) • Food Frequency Questionnaire (FFQ) • Food records • Biomarkers 	<ul style="list-style-type: none"> • Collection of fecal samples at two time points (baseline and after 1 year of intervention). • Extraction of microbial DNA from fecal samples using standard laboratory methods. • Analysis of microbiota composition using 16S rRNA gene sequencing. • Processing of sequencing data into Operational Taxonomic Units (OTUs). • Taxonomic assignment of OTUs at the phylum, genus, and species levels. • Calculation of relative abundance for each bacterial taxon.

Author, year	Country	Study design	Study population and baseline characteristics	Intervention	Methods for measuring dietary adherence	Gut microbiota analysis
				<p>Species</p> <ol style="list-style-type: none"> <i>Parabacteroides distasonis</i> <ul style="list-style-type: none"> Fold change: 2.32 ± 0.62 <i>P-value</i>: 0.025 <i>Faecalibacterium prausnitzii</i> <ul style="list-style-type: none"> Fold change: 1.53 ± 0.33 <i>P-value</i>: 0.516 <i>Bacteroides ovatus</i> <ul style="list-style-type: none"> Fold change: 0.78 ± 0.20 <i>P-value</i>: 0.236 <i>Bacteroides plebeius</i> <ul style="list-style-type: none"> Fold change: 1.56 ± 0.67 <i>P-value</i>: 0.836 <i>Bacteroides uniformis</i> <ul style="list-style-type: none"> Fold change: 0.83 ± 0.21 <i>P-value</i>: 0.667 <i>Coprococcus eutactus</i> <ul style="list-style-type: none"> Fold change: 1.51 ± 0.45 <i>P-value</i>: 0.469 		<ul style="list-style-type: none"> Intrasubject normalization of data to reduce inter-individual variability. Statistical analysis of microbiota changes using repeated-measures ANOVA.
Meslier et al 2020	Italy, France, Denmark	Parallel, randomized controlled trial (RCT) for 8 weeks baseline	<p>82 subject overweight/obesity, 43 male, 39 female age 43 ± 12 year, BMI 31.1 ± 4.5 kg/m²</p> <p>Increase component in ConD</p>	<p>Decrease of: (Baseline -> 4w -> 8w)</p> <ul style="list-style-type: none"> Plasma Carnitin $\downarrow 14\% \rightarrow \downarrow 11\%$ Urin Carnitine $\downarrow 75\% \rightarrow \downarrow 51\%$ LDL 2.90 ± 0.13 mmol/L (base)-> 2.66 ± 0.12 mmol/L (4w) 	<ul style="list-style-type: none"> 7-day food diary Physical activity questionnaire Mediterranean Diet adherence score Blood lipids TMAO (plasma & urine) 	<p>a. Sample Collection</p> <ul style="list-style-type: none"> Fecal samples were collected at baseline, week 4, and week 8 of the intervention.

Author, year	Country	Study design	Study population and baseline characteristics	Intervention	Methods for measuring dietary adherence	Gut microbiota analysis
		vs 4 weeks and 4 weeks vs 8 weeks Comparison: regular diet	<ul style="list-style-type: none"> Propionylcarnitine → meat biomarker L-acetylcarnitine 3-hydroxyisovaleroylcarnitine Indoxyl sulphate → dysbiosis marker Leucine & isoleucine breakdown products Propionylcarnitine / methyl-choline 	<ul style="list-style-type: none"> HDL → 1.26 ± 0.05 mmol/L (base) → 1.18 ± 0.04 mmol/L (4w) Increase component in MedD: <ol style="list-style-type: none"> 3-(3,5-dihydroxyphenyl)propanoic acid–glucuronide → wholegrain biomarker Dihydroxybenzoic acid–glucuronide Oxindole-3-acetic acid → vegetable/fruit intake Urolithin A–glucuronide → nuts biomarker Tryptophan betaine → legume biomarker Pyrogallol-sulphate Methoxy–pyrogallol–sulphate 3,5-dihydroxyphenylpropionic acid Gene richness $5,551,310.5 \pm 120,191$ (stable) MSP richness of 230.9 ± 53.1 (stable) Decrease in microbiome similarity only in MedD Differentially abundant MSPs increased markedly in the MedD group: <ul style="list-style-type: none"> ✓ Baseline: 27 MSPs ✓ Week 4: 77 MSPs ✓ Week 8: 44 MSPs 	<ul style="list-style-type: none"> Carnitine, choline, betaine Gastrointestinal peptides (ghrelin, GLP-1, GIP, insulin, leptin, dsb.) Urinary polyphenols: LC–MS/MS 	<p>b. DNA Extraction & Library Preparation</p> <ul style="list-style-type: none"> Microbial DNA was extracted from fecal samples. Extracted DNA was used to prepare metagenomic sequencing libraries. <p>c. Whole-Genome Shotgun Metagenomic Sequencing</p> <ul style="list-style-type: none"> Sequencing was performed using the Ion Proton Sequencer (ThermoFisher). Each sample generated a minimum of 20 million high-quality reads, with a read length of 150 bp. <p>d. Microbial Composition Analysis</p> <ul style="list-style-type: none"> Taxonomic profiling was conducted using the 9.9-million gene reference catalogue.

Author, year	Country	Study design	Study population and baseline characteristics	Intervention	Methods for measuring dietary adherence	Gut microbiota analysis
				<p>Decrease Gut Microba (4w)</p> <ul style="list-style-type: none"> • <i>Ruthenibacterium lactatiformans</i> • <i>Flavonifractor plautii</i> • <i>Parabacteroides merdae</i> • <i>Ruminococcus torques</i> • <i>Ruminococcus gnavus</i> • <i>Streptococcus thermophilus</i> <p>Increase Gut Microba (4w)</p> <ul style="list-style-type: none"> • <i>Faecalibacterium prausnitzii</i> → anti-inflammatory, butyrate producer • <i>Roseburia</i> → SCFA producer • <i>Lachnospiraceae</i> → fiber fermenter, SCFA producer <p>Increase GMM:</p> <ul style="list-style-type: none"> • Amino acid degradation pathways • Carbohydrate degradation pathways • Triglyceride and glycoprotein degradation • Conversion of acetyl-CoA & glutamate → crotonyl-CoA → precursor butyrate metabolism 		<ul style="list-style-type: none"> • Microbial species were identified through Metagenomic Species Pangenomes (MSP). • Microbial diversity was quantified using: <ul style="list-style-type: none"> ○ Gene richness ○ MSP richness e. Functional Microbiota Analysis • Functional potential was analyzed using the FAnToMet pipeline. • Gut Metabolic Modules (GMMs) were assessed, including pathways related to: <ul style="list-style-type: none"> ○ Amino acid degradation ○ Carbohydrate degradation ○ Butyrate precursor pathways
Tagliamonte et al., 2021	Italy (University of)	Randomized controlled trial	✓ 82 healthy overweight or obese men and women aged 20–65 years with a	MD consumption decreased:	7-day food diary, biweekly evaluation, Italian	Shotgun metagenomics sequencing (Ion Proton

Author, year	Country	Study design	Study population and baseline characteristics	Intervention	Methods for measuring dietary adherence	Gut microbiota analysis
	Naples) & France (Université Paris-Saclay)	(parallel, 8 weeks)	<p>BMI ≥ 24 kg/m² who habitually consumed a low-intake diet of fruits and vegetables (≤ 3 servings/day), no probiotics, functional foods, or dietary supplements, and no more than two portions of whole-grain or fiber-enriched foods per day. All participants had a sedentary lifestyle (MET < 700 min/week).</p> <p>✓ Baseline comparison control group vs mediterranean diet group:</p> <p>✓ No significant effect of sex was observed on baseline plasma concentrations of OEA, LEA, PEA, AEA, and 2-AG, nor on the OEA/PEA and OEA/AEA ratios in either the CT or MD groups.</p>	<ul style="list-style-type: none"> Plasma AEA concentrations after 8 weeks were lower than baseline (-9.1%; $p = 0.02$). The HOMA index decreased after 4 weeks (-26.2%; $p = 0.01$) and 8 weeks (-23.9%; $p = 0.01$). <p>MD consumption increased:</p> <ul style="list-style-type: none"> Significant increases were observed in the OEA/PEA ratio (5.9%; $p = 0.009$) and the OEA/AEA ratio (14.6%; $p = 0.006$), recorded after 4 and 8 weeks of MD consumption, respectively. Significant increases in several MSP species, including <i>Roseburia faecis</i> and <i>R. hominis</i>, were observed after both 4 and 8 weeks. An increase in fiber-degrading bacteria, such as <i>Faecalibacterium prausnitzii</i> and several members of the <i>Lachnospiraceae</i> family, was also detected. Levels of <i>Akkermansia muciniphila</i> were significantly higher in the MD group after 8 weeks of MD consumption. 	Mediterranean Index (IMI) score	platform) for gut microbiome profiling; MSP (Metagenomic Species Pangenome) reconstruction; qPCR for <i>Akkermansia muciniphila</i> quantification
Haro, 2017	Spain	A sub-study of the CORDIOPREV trial (a prospective,	A total of 106 male patients with coronary heart disease (CHD) were included and classified into three groups:	<p>Increased:</p> <ul style="list-style-type: none"> The abundance of the genera <i>Bacteroides</i>, <i>Prevotella</i>, and <i>Faecalibacterium</i> increased in the MetS-OB group after 2 years of 	<p>-A validated 14-item questionnaire was used to assess adherence to the Mediterranean Diet.</p> <p>- A 9-point score was used to</p>	<ul style="list-style-type: none"> Fecal samples were collected and stored frozen at -80°C.

Author, year	Country	Study design	Study population and baseline characteristics	Intervention	Methods for measuring dietary adherence	Gut microbiota analysis
		randomized, open-label, controlled study).	<p>-MetS-OB: 33 obese patients with Metabolic Syndrome (meeting all 5 NCEP-ATP III criteria);</p> <p>-NonMetS-OB: 32 obese patients without Metabolic Syndrome (≤ 2 criteria);</p> <p>-NonMetS-NonOB: 41 non-obese subjects without Metabolic Syndrome.</p> <p>- Age: 59–64 years</p> <p>- BMI: MetS-OB ≈ 32.4 kg/m²; NonMetS-OB ≈ 32.9 kg/m²; NonMetS-NonOB ≈ 27.0 kg/m²</p> <p>- Waist circumference: MetS-OB ≈ 114 cm; NonMetS-OB ≈ 109 cm; NonMetS-NonOB ≈ 97 cm</p> <p>- HOMA-IR was higher in the MetS-OB group</p> <p>- HDL-C was lower and triglycerides were higher in the MetS-OB group compared with the other two groups.</p>	<p>both MED and LF diet consumption compared with baseline ($P < 0.001$, $P < 0.001$, and $P = 0.001$, respectively).</p> <ul style="list-style-type: none"> The relative abundance of the phylum <i>Bacteroidetes</i> increased in the MetS group after 2 years of dietary intervention. However, this increase was statistically significant following the LF diet ($P < 0.001$), whereas only a trend toward an increase was observed with the MED diet. Two years of MED diet consumption increased the abundance of the genera <i>Roseburia</i> and <i>Ruminococcus</i>, as well as the bacterial species <i>P. distasonis</i> and <i>F. prausnitzii</i> ($P = 0.004$, $P = 0.009$, $P = 0.014$, and $P = 0.043$, respectively). In contrast, the LFHCC diet did not affect the abundance of these genera and species. <p>Decreased:</p> <ul style="list-style-type: none"> A significant reduction in the <i>Firmicutes/Bacteroidetes</i> ratio was observed after consumption of the LF diet ($P < 0.001$), whereas only a downward trend was observed with the MED diet. 	<p>evaluate adherence to the Low-Fat Diet.</p> <p>- Dietary fiber intake was calculated using the Spanish Food Composition Tables and a validated FFQ</p>	<ul style="list-style-type: none"> DNA was extracted using the QIAamp DNA Stool Mini Kit. 16S rRNA sequencing (V4 region) was performed using the Illumina MiSeq platform. Bioinformatic processing was conducted with QIIME, with OTU clustering at 97% similarity using the Greengenes database. Alpha diversity (Chao1, Faith's PD), beta diversity (UniFrac), and relative taxonomic abundances were analyzed

Author, year	Country	Study design	Study population and baseline characteristics	Intervention	Methods for measuring dietary adherence	Gut microbiota analysis
				<ul style="list-style-type: none"> Two years of LF diet consumption reduced the abundance of the genera <i>Streptococcus</i> and <i>Clostridium</i> ($P = 0.010$ and $P < 0.001$, respectively), while MED diet consumption did not affect the abundance of these genera. Triglyceride (TG) levels decreased after 2 years of follow-up in the MetS-OB group ($P < 0.001$) following consumption of both diets. 		
Choo et al 2023	Australia	A randomized controlled trial (RCT) with a 2 × 2 cross-over design. Each participant completed two intervention phases: the MedDairy diet and the low-fat diet (Low-Fat Diet/LFD), each lasting 8 weeks, separated by an 8-week washout period.	<p>The study population consisted of adults aged 45–75 years who were at risk of cardiovascular disease (CVD). Eligible participants had a systolic blood pressure ≥ 120 mmHg, were not taking antihypertensive medication, and met at least two additional cardiometabolic risk factors, including:</p> <ul style="list-style-type: none"> -Overweight or obesity (BMI ≥ 25 kg/m²) - Increased waist circumference (>94 cm for men; >80 cm for women) - Dyslipidaemia: <ul style="list-style-type: none"> - Total cholesterol ≥ 5.5 mM - Triglycerides ≥ 2.0 mM 	<ul style="list-style-type: none"> - An 8-week Mediterranean diet supplemented with dairy foods (MedDairy) did not significantly change overall gut microbiota structure or diversity compared with a low-fat control diet. - The MedDairy intervention altered the relative abundance of specific gut bacteria, including: <ul style="list-style-type: none"> • An increase in <i>Butyricicoccus</i> • A decrease in <i>Collinsella</i> and <i>Veillonella</i> -Changes in <i>Butyricicoccus</i> were associated with cardiovascular outcomes: 	<p>Dietary adherence was assessed with a 14-item Mediterranean Diet adherence tool and a 9-item low-fat diet adherence tool, which were completed by participants every two weeks during the respective intervention phases. In addition, a 3-day weighed food record (WFR) was obtained to calculate the Mediterranean Diet Score (MDS) using 10-point and 18-point scoring systems. These measures indicated that participants complied with both diets, with higher compliance to the Mediterranean diet during the MedDairy phase.</p> <p>Samples were collected using</p>	<p>Faecal samples were collected using OMNIgene GUT kits and stored at -80 °C within 24 hours. DNA was extracted using the DNeasy PowerLyzer PowerSoil Kit, and total bacterial load was confirmed by quantitative PCR. The 16S rRNA V4 region was amplified and sequenced on an Illumina MiSeq platform. Sequence data were processed in QIIME2 for demultiplexing, taxonomic assignment (SILVA 138), and</p>

Author, year	Country	Study design	Study population and baseline characteristics	Intervention	Methods for measuring dietary adherence	Gut microbiota analysis
			<ul style="list-style-type: none"> - LDL cholesterol ≥ 3.5 mM - Low HDL (≤ 0.9 mM for men; ≤ 1.0 mM for women) - Impaired glucose tolerance (6.1–7.8 mmol/L) - Family history of CVD or type 2 diabetes - Individuals consuming medicinal doses of calcium or >1000 mg/day omega-3 supplements were excluded. - Average home systolic blood pressure: 128 mmHg. - Mean BMI: approximately 30.5 kg/m² (obesity category). - Fasting glucose: 5.6 mmol/L. - Total cholesterol: 5.7 mmol/L. - Majority were women (24 out of 34 participants). - Average education level: 16 years. - There were no significant differences between groups at baseline, except for slightly higher glucose levels in one group. 	<ul style="list-style-type: none"> • Increased <i>Butyricoccus</i> was inversely correlated with systolic blood pressure • Increased <i>Butyricoccus</i> was positively correlated with fasting glucose levels • These associations were observed only during the MedDairy intervention, not during the low-fat diet <p>-Higher adherence to the Mediterranean diet was positively associated with greater abundance of <i>Butyricoccus</i>.</p> <p>- No significant associations were found between gut microbiota changes and anthropometric measures (e.g., BMI, body composition) or cognitive function.</p> <p>- Overall, the findings indicate that the MedDairy diet can modulate specific gut bacterial taxa linked to cardiovascular risk, even without major changes in overall gut microbiota composition.</p>	<p>OMNIgene GUT kits and stored at -80°C within 24 h. DNA was isolated using the DNeasy PowerLyzer PowerSoil Kit and the total bacterial load was confirmed by quantitative PCR. The 16S rRNA V4 region was amplified and sequenced on an Illumina MiSeq platform. Sequence data were processed in QIIME2 for demultiplexing, taxonomic assignment (SILVA 138) and diversity analyses, with all samples rarefied to 7794 reads. Microbiota profiling was performed by calculating alpha diversity (richness, evenness, phylogenetic diversity) and weighted UniFrac distances.</p> <p>Functional Capacity and Microbial Metabolite Several included studies assessed microbial functional characteristics and metabolic-related markers (23). Meslier et al. reported changes in microbial gene richness and microbial functional profiles after Mediterranean Diet intervention. Tagliamonte et al.</p>	<p>diversity analyses, with all samples rarefied to 7,794 reads. Alpha diversity (richness, evenness, phylogenetic diversity) and weighted UniFrac distances were computed for microbiota profiling.</p>

Author, year	Country	Study design	Study population and baseline characteristics	Intervention	Methods for measuring dietary adherence	Gut microbiota analysis
					reported changes in microbial taxa and metabolic markers related to endocannabinoid- associated pathways.	

Assessing Risk of Bias

The risk of bias assessment was conducted using the Cochrane Risk of Bias 2 (RoB 2) tool for randomized controlled trials. Although five publications met the eligibility criteria, the included reports did not represent five independent study populations. Meslier et al. and Tagliamonte et al. reported outcomes from the same cohort of 82 participants, while Haro et al. (2016) and Haro et al. (2017) were derived from the CORDIOPREV study population. Therefore, these reports were considered as related evidence from shared cohorts rather than independent datasets during the interpretation of the risk of bias and overall certainty of evidence. The RoB 2 assessment was performed across five domains: bias arising from the randomization process, bias due to deviations from intended interventions, bias due to missing outcome data, bias in measurement of the outcome, and bias in selection of the reported result. Each domain was classified as low risk of bias, some concerns, or high risk of bias according to the RoB 2 guidance.

Overall, the included reports demonstrated variable risk of bias profiles. Most assessments indicated low risk of bias or some concerns; however, limitations related to reporting transparency and methodological details were identified in several domains. These limitations were considered when interpreting the findings, particularly because the certainty of evidence for the reported outcomes was assessed as low to very low. The risk of bias findings was interpreted together with the certainty of evidence assessment to avoid overestimation of the strength of the available evidence. The detailed RoB 2 judgements for each included report are presented in Figure 2.

Unique ID	Study ID	Experimental	Comparator	Outcome	Weight	D1	D2	D3	D4	D5	Overall
MD01	Haro et al., 2016	Mediterranean diet (Med Low-fat, high-complex car	Insulin sensitivity index	1		+	+	+	+	+	+
MD02	Meslier et al., 2020	Mediterranean diet (Med Habitual diet (ConD), parti	Plasma LDL-cholesterol	1		+	+	+	+	+	+
MD03	Tagliamonte et al.,	Mediterranean diet (isoc: Control (habitual diet, part	Plasma endocannabinoids	1		+	+	+	+	!	+
MD04	Haro et al., 2017	Mediterranean diet (35% Low-fat diet (<30% fat: <1	Gut microbiota composition	1		+	+	+	+	!	+
MD05	Wade et al., 2023	MedDairy diet → Mediter Low-fat diet (control).	Primary: Systolic and di	1		+	+	+	+	!	+

Figure 2. Risk of bias summary for mediterranean diet intervention studies

Synthesis of Results

Five publications from approximately three independent cohorts were included in the synthesis of results. Due to substantial heterogeneity in participant characteristics, intervention duration, dietary comparators, microbiome analytical methods, and reported outcomes, a meta-analysis was not performed. Therefore, the findings were synthesized narratively (Table 3).

The included reports evaluated different Mediterranean Diet approaches, including conventional Mediterranean Diet patterns, Green-Mediterranean Diet, and Mediterranean Diet supplemented with dairy products (MedDairy). In general, the included studies reported changes in selected characteristics of the gut microbiota and metabolic-related outcomes, but the magnitude and consistency of these findings differed among studies. These findings should be interpreted with caution given the small number of independent cohorts, overlapping study populations and the low to very low certainty of the evidence.²³

Gut Microbiota Composition

Composition of the Gut Microbiota The included studies reported changes in specific bacterial taxa following Mediterranean Diet interventions, however, no consistent changes were identified across all studies. Several bacterial genera were changed by Haro et al. after a long-term dietary intervention, including an increase in abundance of *Roseburia* and *Parabacteroides distasonis*. Meslier et al. reported changes in the microbial composition following an 8-week Mediterranean Diet intervention, including changes in taxa such as *Faecalibacterium prausnitzii*, *Lachnospiraceae*, and *Roseburia*, and reductions in certain taxa associated with an altered microbial profile.

Tagliamonte et al., using participants from the same cohort as Meslier et al., reported increased abundance of *Akkermansia muciniphila* following Mediterranean Diet intervention. Choo et al. reported that the MedDairy intervention did not significantly modify overall gut microbiota structure or diversity compared with the control diet but was associated with changes in specific taxa, including increased abundance of *Butyricicoccus*. Although some studies showed differences in bacteria related to fiber fermentation and pathways related to short-chain fatty acids, the differences in study design, sequencing method and reported results did not allow for a direct comparison of their outcomes.²⁴

Table 3. Summary of Findings (GRADE) for Mediterranean Diet Interventions on Gut Microbiota and Cardiometabolic Outcomes

Outcome	Number of studies/reports	Participants / cohorts	Summary of findings	Certainty of evidence (GRADE)	Reasons for downgrading
Gut microbiota composition (changes in bacterial taxa and microbial diversity)	5 reports from approximately 3 independent cohorts	Adults with overweight, obesity, metabolic risk factors, or cardiovascular risk factors	Mediterranean Diet interventions were associated with changes in selected bacterial taxa, including SCFA-associated taxa such as <i>Faecalibacterium</i> , <i>Roseburia</i> , <i>Lachnospiraceae</i> , and <i>Butyricicoccus</i> . However, microbial changes were heterogeneous across studies due to differences in intervention duration, sequencing methods, and participant characteristics.	Low	Downgraded due to risk of bias, inconsistency between studies, and imprecision caused by small sample sizes and limited independent cohorts.
SCFA-related microbial pathways (butyrate-producing bacteria and microbial functional characteristics)	3 reports	Approximately 3 independent cohorts	Several studies reported increased abundance of bacteria associated with SCFA production, particularly <i>Faecalibacterium</i> and <i>Roseburia</i> . However, most studies inferred SCFA-related effects from bacterial taxa rather than directly measuring SCFA concentrations.	Very Low	Downgraded due to indirectness (surrogate microbial markers instead of direct metabolite measurements), inconsistency, and imprecision.
<i>Akkermansia muciniphila</i> abundance	2 reports from the same cohort	82 overweight/obese participants	Mediterranean Diet intervention was associated with increased abundance of <i>Akkermansia muciniphila</i> . However, findings were based on a single cohort and measured using different analytical approaches.	Very Low	Downgraded due to risk of bias, imprecision, and limited generalizability because evidence originated from overlapping reports of the same cohort.
Cardiometabolic outcomes (lipid parameters, insulin-related markers, inflammatory markers)	4 reports	Approximately 3 independent cohorts	Mediterranean Diet interventions were associated with changes in selected cardiometabolic markers, including lipid profiles, insulin-related parameters, and cardiovascular-related indicators. However, associations between microbiota changes and clinical outcomes remain uncertain.	Low	Downgraded due to risk of bias, inconsistency, small sample sizes, and uncertainty regarding causal relationships between microbiota changes and clinical outcomes.

Functional Capacity and Microbial Metabolites

Some studies examined the microbial functional characteristics and metabolic-related markers.²⁵ Meslier et al. reported modulation of microbial gene richness and microbial functional profiles following Mediterranean Diet intervention.²⁰ Tagliamonte et al. described changed microbial taxa and metabolic markers associated with endocannabinoid-related pathways.²¹ Haro et al. described changes in microbial composition associated with metabolic pathways related to carbohydrate metabolism and microbial metabolites.¹⁸ However, methodological differences and heterogeneity in outcome measures precluded meta-analysis across studies. These results suggest possible effects of Mediterranean Diet interventions on microbial functional features. Nevertheless, the available evidence is still limited due to the small number of independent cohorts and methodological heterogeneity.

Clinical Outcomes

Several of the reports included also assessed microbiome changes in relation to cardiometabolic outcomes. Meslier et al. described changes in parameters related to lipids after a Mediterranean Diet intervention. They reported changes in metabolic markers including insulin-related parameters and endocannabinoid-associated metabolites Tagliamonte et al. Haro et al. reported changes in metabolic profiles in participants with cardiovascular risk factors after dietary interventions. During the MedDairy intervention, Choo et al. reported associations between changes in *Butyricicoccus* abundance and selected cardiovascular-related parameters. However, the link between microbiota changes and cardiometabolic outcomes remains unclear, as most of the associations reported were drawn from individual studies and were not consistently replicated across independent cohorts

Integrated Findings

To summarize, the studies reviewed suggest that Mediterranean Diet interventions may affect specific features of gut microbiota composition and microbial functional features. However, the data available do not allow for firm conclusions about the magnitude or clinical relevance of these effects. In interpreting the findings, the small number of independent cohorts, overlap between published reports, heterogeneity of intervention protocols, and low to very low certainty of the evidence need to be considered. Further well-designed randomized controlled trials using standardized methods for microbiome assessment are needed to better understand the relationship between Mediterranean Diet interventions, modulation of gut microbiota and cardiometabolic outcomes.

DISCUSSION

Primary Findings

This systematic review evaluated the available evidence regarding the association between Mediterranean Diet interventions and changes in gut microbiota composition and cardiometabolic-related outcomes among individuals with obesity or metabolic risk factors. The included evidence consisted of five publications derived from approximately three independent cohorts, as some reports originated from overlapping study populations.

Overall, the included studies suggested that Mediterranean Diet interventions may impact selected aspects of gut microbiota composition, including shifts in specific bacterial taxa implicated in the short-chain fatty acid (SCFA) metabolism.²⁶ However, the size and stability of these changes were inconsistent across studies because of differences in participant characteristics, duration of intervention, dietary comparators and microbiome analytical approaches. Several studies reported increased abundance of taxa associated with SCFA production such as *Faecalibacterium prausnitzii*, *Roseburia*, *Lachnospiraceae* and *Butyricicoccus*. However, these findings should be interpreted with caution as the certainty of evidence was rated low to very low and most outcomes were surrogate markers of the microbiome rather than long-term clinical endpoints.

Gut Microbiota Composition

The included studies reported modulation of gut microbial composition following Mediterranean Diet interventions, but the specific microbial signatures varied across studies.²⁷ Several reports identified increases in bacterial taxa commonly associated with fiber fermentation and SCFA-related pathways, including *Faecalibacterium prausnitzii* and *Roseburia*.²⁸ Meslier et al. reported alterations in the microbial composition following an 8-week intervention with the Mediterranean Diet, including changes in taxa associated with microbial metabolic functions. Tagliamonte et al. (2021),²¹ who analyzed participants from the same cohort, found increased abundance of *Akkermansia muciniphila* following dietary intervention.

Haro et al. reported longer-term dietary intervention and changes in microbial taxa in the CORDIOPREV cohort including changes in bacterial genera associated with metabolic pathways. However, differences in sequencing platforms, taxonomic resolution and analytical pipelines prevented direct comparison between studies. Thus, while available evidence suggests Mediterranean Diet interventions have the potential to modify gut microbial composition, the extent to which such changes are consistent biological responses is unclear.²⁹

Short Chain Fatty Acid Related Pathways

Effects related to SCFA were mostly inferred from changes in the abundance of bacterial taxa associated with SCFA production, and not from direct measurement of SCFA concentrations in the majority of the included studies.³⁰ Butyrate-producing taxa, such as *Faecalibacterium* and *Roseburia*,

were found to be enriched after Mediterranean Diet interventions.³¹ The specific bacterial taxa associated with SCFA pathways, however, differed between studies and were not functionally validated. Uncertainty about the size of these effects was driven by differences in microbiome analysis methods, sample size and outcome definitions. These findings suggest a potential role of Mediterranean Diet interventions to modulate microbial pathways involved in SCFA metabolism, although more robust evidence from standardized functional microbiome analyzes is warranted.

***Akkermansia muciniphila* and Microbial Diversity**

Two included studies assessed changes in abundance of *Akkermansia muciniphila* following Mediterranean Diet interventions.³² Both showed increased abundance after dietary intervention, but differences in sequencing approaches and taxonomic resolution limited direct comparison.³³ Changes in microbial diversity were also reported in some studies, including alterations in alpha and beta diversity measures. Nevertheless, diversity outcomes varied according to analytical methods and sequencing approaches. Due to the limited number of studies and methodological heterogeneity, the certainty of evidence for changes in *Akkermansia muciniphila* abundance and microbial diversity remains very low.

Cardiometabolic Outcomes

These were change in lipid parameters, inflammatory markers and insulin related outcomes.³⁴ Meslier et al. and Tagliamonte et al. reported changes in metabolic markers after short term dietary intervention, while Haro et al. reported metabolic changes among individuals with cardiovascular risk factors. Choo et al. found associations between changes in some bacterial taxa and cardiovascular-related parameters during the MedDairy intervention. However, the relationship between microbiota alterations and cardiometabolic improvements remains uncertain.³⁵ Most findings were based on individual studies with limited sample sizes, and the available evidence does not establish whether microbiota changes directly mediate clinical outcomes.

Limitations

This systematic review has several limitations. First, the number of eligible randomized controlled trial reports was limited, and some publications originated from the same underlying cohorts, creating potential risk of double-counting if interpreted as independent studies. Therefore, findings should be considered in the context of approximately three independent cohorts rather than five separate populations. Second, there was a lot of heterogeneity in terms of intervention duration, dietary adherence assessment, microbiome sequencing methods, and outcome definitions, which hindered cross-study comparability and prevented quantitative synthesis. Third, most of the studies included in the review were conducted in European populations, and thus the generalizability of the findings to other populations with different dietary patterns and microbiome characteristics is limited. Many of the reported outcomes were surrogate markers rather than long-term clinical outcomes, for

example changes in microbial composition and metabolic biomarkers. Finally, the certainty of evidence for several outcomes was low or very low indicating that further well-designed studies are needed before any firm conclusions can be drawn.

Clinical and Research Implications

The results of this systematic review show that interventions with the Mediterranean Diet could influence some components of the gut microbiota composition and the microbial metabolic pathways. However, the clinical relevance of these microbiome alterations remains unclear, given the limitations in the quality of evidence to date. Future studies should focus on larger RCTs with longer follow-up, standardized methods for microbiome analysis, and combined assessment of microbiome composition, microbial metabolites, and clinical outcomes.²⁸ These approaches could help to clarify if microbiota modulation is a relevant mechanism underlying the metabolic effects related to Mediterranean Diet adherence.

Future Directions

Future RCTs should:

1. Standardize adherence and outcome measure metrics (validated scores, biomarker verification).
2. Integrate multi-omic approaches (metagenomics, metabolomics, transcriptomics, bile acid profiling) to improve causal inference.
3. Broaden trial design and populations to diverse ethnic and dietary contexts, with precision-nutrition stratification by baseline microbiota/metabolic phenotypes.
4. Other clinical endpoints rather than surrogate markers (such as cardiovascular events and patient-centered outcomes).

CONCLUSION

This systematic review suggests that Mediterranean Diet interventions may influence selected aspects of gut microbiota composition and microbial functional characteristics among individuals with obesity and metabolic risk factors. The included evidence indicates potential changes in SCFA-associated bacterial taxa, microbial diversity, and selected cardiometabolic-related outcomes; however, the magnitude and consistency of these effects remain uncertain.

Interpretation of these findings should consider important limitations, including the small number of available reports, overlapping study populations, methodological heterogeneity across microbiome assessment approaches, and the predominance of surrogate outcomes rather than long-term clinical endpoints. Therefore, the current evidence does not allow definitive conclusions regarding the causal role of Mediterranean Diet-induced microbiota changes in improving metabolic health.

Future randomized controlled trials with larger populations, standardized microbiome analysis methods, longer follow-up periods, and integrated assessment of microbial composition, metabolites, and clinical outcomes are needed to clarify the clinical relevance of Mediterranean Diet-associated microbiota modulation.

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Ethical Statement

Ethical approval was not required as this study used published data. The protocol was registered in PROSPERO (CRD420251155212).

Conflict of Interest

The authors declare that they have no competing interests.

Author Contributions

All authors participated in the manuscript preparation and approved the final manuscript.

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