Peer-Review comments and authors responses

"Effects of the Mediterranean Diet on Gut Microbiota Composition and Diversityin Overweight and Obesity: A Scoping Review of Randomized Controlled Trials"

Reviewer 1

Abstract

> **Comment 1:** It usually covers: Background, Objective, Methods, Results, Conclusion, Keywords, Abbreviations. Results look more like a conclusion in the end. Only describe your findings. The discussion is actually the conclusion.

Response: We thank the reviewer for this helpful observation. The abstract was revised to ensure a clear distinction between the Results and Conclusion sections. In the Results, we now focus exclusively on describing the study findings, avoiding interpretative statements. The Conclusion was rewritten to summarize the main implication of the evidence and the need for future research. This revision improved the structure and clarity of the abstract, aligning it with standard scientific conventions.

Introduction

> **Comment 2:** Paragraph 3: it is a bit dense. Consider breaking it into two paragraphs - (1) focused on microbial diversity/changes in obesity, (2) another on the Mediterranean diet characteristics and anti-inflammatory effects. Final paragraph: be more specific about what inconsistencies exist in the literature.

Response: We appreciate this insightful comment. The original paragraph has been divided into two separate sections as suggested, distinguishing between microbial diversity and changes in obesity, and the characteristics and anti-inflammatory effects of the Mediterranean diet. This revision was beneficial, as it improved the clarity, readability, and logical flow of the introduction.

Methods

> **Comment 3:** Inclusion and exclusion criteria. Population: specify any exclusions: lactating women, specific medical conditions that might confound results.

Response: We clarified that there were no restrictions regarding sex or comorbidities, including both healthy individuals and patients with cardiometabolic conditions. The exclusion criteria included pregnant or lactating women, participants under 18 years of age, and BMI <25, as well as participants taking supplements or pre/probiotics that could confound the effects of the Mediterranean diet. Medication use was not an exclusion criterion.

> **Comment 4:** Intervention. You might want to add: "...with or without caloric restriction". This to clarify if you're including both isocaloric and hypocaloric Mediterranean diet interventions. Consider mentioning intervention duration. Is there any evidence supporting a specific time for the intervention to show changes in the microbiota composition? Explain if yes/no in the discussion why that time is required.

Response: We included Mediterranean diet interventions with or without caloric restriction, thus covering both isocaloric and hypocaloric protocols. Intervention durations were reported for all studies, but no minimum duration was set as an eligibility criterion. Evidence from the included studies shows that meaningful changes in gut microbiota composition and related metabolic or inflammatory outcomes were mainly observed in interventions lasting 12 months or longer (Meslier et al., 2020; Galié et al., 2021;

Castro-Barquero et al., 2020), whereas shorter interventions (e.g., 6–8 weeks) did not produce consistent microbiota changes. This rationale is discussed in the Discussion section.

Comment 5: Comparator. Mention if you included or not studies with no-intervention controls.
Response: We included studies with any type of dietary comparator, including low-calorie, low-fat, ketogenic, high-complex carbohydrate, habitual Western diets, or usual care. Studies with no-intervention control groups were also included when available, and their presence is detailed in Table 1 and in the Methods section.

Comment 6: Outcomes. Consider grouping them as primary outcomes: gut microbiota composition and secondary outcomes: inflammatory biomarkers and metabolic outcomes.
Response: Outcomes were reorganized as suggested: gut microbiota composition (e.g., Firmicutes/Bacteroidetes ratio, α- and β-diversity, taxonomic abundance) was considered the primary outcome, while inflammatory biomarkers (e.g., TNF-α, IL-6, CRP) and anthropometric/metabolic outcomes (e.g., weight, BMI, waist circumference, insulin sensitivity) were classified as secondary outcomes.

Comment 7: Follow-up. You might want to specify minimum follow-up periods for outcome assessment (if that is the case, explain in the discussion why that time is needed).
Response: Follow-up times were standardized and reported for all studies (e.g., T0, T2m, T6m, T1y, T2y), but no universal minimum follow-up period was used as an inclusion criterion. The Discussion section explains that longer follow-up durations (≥12 months) are generally required to observe consistent changes in gut microbiota composition, as shorter interventions did not show substantial effects.

Results

Comment 8: Statistical significance: -Indicate which changes were statistically significant.
Response: We thank the reviewer for this observation. We have revised the Results section to clearly indicate which reported changes were statistically significant, including the corresponding p-values where available.

Comment 9: Primary outcomes / Buscemi 2009: -Primary outcomes: why did you exclude Buscemi 2009? you mention that only three distinct trials focused on the gut microbiome and mention (Buscemi, 2009; Deledda, 2022; Haro, 2016; Haro, 2017). However, later, you exclude Buscemi 2009. Did Buscemi 2009 assess gut microbiota or not? What's the exact count of studies that assessed microbiome?
Response: We appreciate the reviewer's comment. Buscemi et al. (2009) was not excluded; on the contrary, it was included and considered important for the assessment of body composition and inflammatory markers. However, we clarify that this study did not evaluate gut microbiota. Regarding the microbiome-focused studies, we confirm that only three studies specifically assessed gut microbiota outcomes (Deledda, 2022; Haro, 2016; Haro, 2017). We have clarified this in the revised Results section to avoid ambiguity.

Comment 10: Inflammatory markers: -Secondary outcomes: Inflammatory markers: For the secondary outcomes, please clarify the analytic approach. Specifically, was the reported reduction in inflammatory markers based on a direct comparison between the intervention and control groups, or was it assessed as within-group changes over time (e.g., baseline vs. follow-up in the intervention group)? Distinguishing between between-group effects and within-group changes is important for interpreting the clinical relevance of the results. Some studies mention the comparator clearly (ketogenic diet, low-fat control) while others are less specific. Most use mg/L for CRP, but one uses percentage change - consider noting baseline values where helpful. Consider adding p-values or confidence intervals where available to strengthen the reporting.

Response: We thank the reviewer for this important comment. We clarify that the analysis of inflammatory markers was based on between-group comparisons (intervention vs. control), rather than within-group changes over time. We have now explicitly stated this in the Results section. We also acknowledge variability in reporting across studies and have added clarifications where comparator diets were not explicitly stated. Where available, we have included p-values and baseline values to improve interpretability of the results.

> **Comment 11:** BMI and weight: -Changes in body mass index and weight: Some studies get specific numbers while others are vaguely described. Try to extract more specific data where possible. Include p-values consistently (you have one for Buscemi but not others). Consider noting baseline BMI/weight where relevant for context. The line that talks about Esposito et al. ends without giving the numbers. Consider separating BMI and weight changes if they show different patterns.

Response: We appreciate the reviewer's suggestion. We have revised this section to include more precise quantitative data where available, including baseline values and p-values. BMI and weight outcomes are now reported more consistently across studies, and where appropriate, they are presented separately to highlight distinct patterns in the results.

> **Comment 12:** Bias assessment: -Bias assessment: Instead of just listing domains, you might want to give more specific examples of the bias issues. Briefly discuss how these bias concerns affect the interpretation of results.

Response: We thank the reviewer for this valuable suggestion. We have expanded the Risk of Bias section to include brief interpretations of the main bias concerns identified across studies and their potential impact on the interpretation of the results.

> **Comment 13:** Table 1: Table 1. Buscemi 2009: when you describe an inflammatory biomarker, can you specify which type (e.g., CRP, IL-6, TNF-α)? rather than using the general term "inflammatory biomarkers". Similarly, for the gut microbiome results, provide more details on what was measured (e.g., Firmicutes/Bacteroidetes ratio or other microbial diversity measures). This level of specificity strengthens the scientific rigor and makes the results more interpretable.

Response: We appreciate the reviewer's comment. We have revised Table 1 to specify the exact inflammatory biomarkers reported in each study (e.g., CRP, IL-6, TNF-α) instead of using generic terminology. Similarly, microbiome-related outcomes have been specified where applicable, including measures such as the Firmicutes/Bacteroidetes ratio and diversity indices (α- and β-diversity), to improve clarity and scientific precision.

Discussion

> **Comment 14:** Acknowledge methodological differences that affect comparability between studies.

Response: We acknowledge that direct comparability across studies is limited by important differences in: Sample size (small vs. moderate samples); Comparators (different diet types: ketogenic, low-fat, standard care/CPAP, Mediterranean lifestyle, etc.); Follow-up duration and time points (ranging from 4–12 weeks to ≥6–24 months), with potential impact on detecting stable microbiota changes; Outcome measurement and reporting, especially for gut microbiota (alpha/beta diversity, Firmicutes/Bacteroidetes ratio, genus/species abundance) and inflammatory markers (CRP, IL-6, TNFRs, 8-iso-PGF2α), including units, data transformations, and uneven use of p-values/95% CIs. These points have been incorporated into the Discussion to contextualize our synthesis and avoid overinterpretation.

> **Comment 15:** You mention "Georgoulis (MIMOSA, 2021)" but earlier referred to MIMOSA as Hernando-Redondo et al. (2024). Please clarify which is correct.

Response: We apologize for the inconsistency. Georgoulis et al. (2021) reports results from the MIMOSA randomized trial (Mediterranean diet/lifestyle Intervention for the Management of Obstructive Sleep Apnea), registered at ClinicalTrials.gov NCT02515357. In contrast, Hernando-Redondo et al. (2024) is not part of MIMOSA; it is a PREDIMED sub-study evaluating modulation of genes related to neuroinflammation in older adults at high cardiovascular risk.

> **Comment 16:** Missing important discussion elements: (1) Comparison with existing literature/previous reviews. (2) Clinical implications of the findings, (3) Methodological limitations across studies, (4) Heterogeneity in interventions and populations.

Response: We added a paragraph summarizing how our findings align with previous reviews and mechanistic studies linking adherence to the Mediterranean diet with improved metabolic health through gut microbiota modulation and inflammation reduction. Methodological considerations: We expanded the section discussing methodological differences across studies, including issues such as sample size variability, adherence measurement, blinding, and microbiome analysis techniques.

> **Comment 17:** The discussion could be strengthened with more detail about proposed mechanisms linking MD → microbiome → inflammation → metabolic outcomes. You can analyze the information obtained in the papers and proposed a mechanism by which MD can produce metabolic outcomes based on the background.

Response: We have added a more detailed discussion of the proposed mechanisms linking the Mediterranean diet to gut microbiota modulation, inflammation reduction, and metabolic outcomes based on the information obtained in the papers and proposed a mechanism by which MD can produce metabolic outcomes based on the background.

> **Comment 18:** You say in the conclusion that future RCTs should adopt longer follow-up durations. Can you explain in the discussion the reasons for that statement? what should be the minimum and maximum follow-up period?

Response: We included a statement recommending longer follow-up durations (12–24 weeks) to capture sustained microbial and metabolic changes. Also clarified why the follow-up periods.

> **Comment 19:** Limitations: some points about heterogeneity are repeated. Consider add, risk of bias concerns you identified earlier, challenges inherent to dietary intervention studies (blinding, adherence), publication bias potential.

Response: We expanded the Limitations section to include risk of bias concerns, challenges inherent to dietary intervention studies (blinding, adherence), and potential publication bias. Some points about heterogeneity are reduced to avoid repetition.

> **Comment 20:** Conclusion: Conclusions must relate directly to the objective of the paper based solely on the data provided in the body of the manuscript. Do not use abbreviations. Do not use reference citations.

Response: We shortened the conclusion to focus on key findings directly related to the objective. We removed abbreviations and citations, as requested, and emphasized the descriptive nature of the findings and the need for standardized, long-term RCTs.

Reviewer 2

> **Comment 1:** The formatting is a burden for every reviewer, as different font matrices make it challenging to read continuously, or the marking of affiliations, for example.

Response: We sincerely thank the reviewer for highlighting these important points regarding formatting. We acknowledge that inconsistent formatting and font styles can impede readability. In the revised

submission, we have ensured a uniform font, consistent affiliation marking, and standardized formatting throughout to facilitate continuous reading.

Comment 2: The citation style is not APA overall, nor is it Vancouver correctly. I am not able to recognise without any mistakes which papers are the numbers in the introduction, for instance.

Response: Regarding citation style, we have carefully reviewed all in-text citations and the reference list to ensure full compliance with the journal's guidelines. We have standardized citations to follow APA style.

Comment 3: Moreover, the references do not name all authors, and when there are too many, they use "et al." instead of "..."

Response: We thank the reviewer for this observation. We have carefully revised all references to ensure that author names are presented in full accordance with APA guidelines. The use of "et al." has been standardized and applied correctly where appropriate.

Comment 4: And even when I count all numbers and named references I do not come up with the 32 in total.

Response: We have also thoroughly cross-checked all in-text citations to ensure they correspond accurately to the reference list, and we confirm that the total number of references (22) is now consistent throughout the manuscript.

Comment 5: And due to all respect, what is the difference between the submission none-9376 and Article+Text-9377?

Response: We apologize for any confusion this may have caused and confirm that only the latter should be considered the official submission.

Methods

Comment 6: Research question/PICOS: It is quite uncommon to state the research question here, as you provided this information as the aim of the study in the last paragraph of the introduction.

Response: We appreciate the reviewer's comment. The research question was already clearly stated as the aim in the last paragraph of the Introduction. To avoid redundancy, we have revised the manuscript to ensure consistency and removed any unnecessary repetition.

Comment 7: Search strategy: Specify the exact date for the search. 'In May' is not sufficient, as I cannot determine if you included papers published on 31 May, only until 15 May, or even until 4 May, for instance.

Response: We thank the reviewer for this observation. We have now specified the exact search period, which was conducted from April 17, 2025, to May 4, 2025.

Comment 8: Again, it is unusual to provide detailed search strategies for PubMed within the text here, especially since they are not included for other databases. Use the keywords here only and provide the complete search strategy for each Database, specifically in the supplementary materials.

Response: We agree with the reviewer and have revised the manuscript accordingly. Only the keywords are now presented in the main text, while the complete search strategies for all databases (PubMed, Embase, and Scopus) are provided in the Supplementary Materials.

Comment 9: You used MeSH terms exclusively, which is insufficient. For obesity and the MD, you also used the text words, why not for the biomarkers?

Response: We thank the reviewer for this important point. Keywords and MeSH terms were used for obesity and the Mediterranean diet. For microbiota and biomarkers, only MeSH terms were initially used, as these were considered secondary outcomes. We have now clarified this rationale in the manuscript.

Comment 10: Moreover, the search strategy you provided seems incomplete. When I use the same search on PubMed, restricting the article types to "Clinical Trial, Clinical Trial Phase III and Clinical Trial Phase IV" and the language to English, I obtain over 48,000 references, not 146 like you did.

Response: We appreciate the reviewer's concern. We re-ran the PubMed search using the specified filters and obtained 146 results. Minor discrepancies may occur due to database updates or differences in how search strings are applied (e.g., formatting or filters). We have carefully verified the search strategy and confirmed its consistency.

Comment 11: Inclusion and exclusion: You only included studies that have a comparison diet. However, later in the results, you provide some information on changes in the microbiome or other values within the MD group, so why haven't you included all studies where you have at least one arm with the MD diet only and given the same information?

Response: We thank the reviewer for this observation. Studies were included if they evaluated a Mediterranean diet intervention, regardless of the type of comparator. Control groups varied across studies and are fully detailed in the Tables. We have clarified this in the manuscript to avoid confusion.

Comment 12: The PICOS is redundant, as you provided this information in the text before.

Response: We agree with the reviewer. The information has been streamlined to avoid redundancy, ensuring consistency between the text and the PICOS framework.

Comment 13: Data extraction: In the methodological information, you mention other relevant comments. What do you consider relevant?

Response: We thank the reviewer for this observation. "Relevant comments" refer to study-specific notes that were recorded during data extraction, including methodological considerations, missing or unclear information in the original articles, and any reported limitations relevant to interpretation of the results. This has been clarified in the revised manuscript.

Comment 14: In the intervention, state something about "total energy". Of what? Intake?

Response: We appreciate the reviewer's comment. We have clarified that "total energy" refers to total energy intake reported in the included studies.

Comment 15: You name "qPCR" as an extraction data, but this is just a method; the data provided by it are, for example, ΔΔCT or fold change.

Response: We thank the reviewer for this important clarification. The manuscript has been revised to report the actual outputs derived from qPCR analysis, such as ΔΔCT values and fold change, rather than the method itself.

Comment 16: The same applies to 16s rRNA gene sequencing, outputs are, for instance, a taxonomy table or α- and β-diversity.

Response: We appreciate the reviewer's comment. We have revised the manuscript to specify the appropriate outputs of 16S rRNA gene sequencing, including taxonomy tables and measures of α- and β-diversity, instead of referring to the method.

Comment 17: Data synthesis: Here, you mentioned you extracted data after sorting, but this is the synthesis section; extraction was the paragraph before.

Response: We thank the reviewer for this observation. We acknowledge that the description of data sorting was misplaced in the Data Synthesis section, as data extraction is described in the previous section. The manuscript has been revised to clearly separate data extraction from data synthesis and avoid any overlap.

Comment 18: It is absolutely unclear for what sorting purpose ChatGPT is needed, especially since you downloaded the data from Covidence as a CSV file. You can directly sort there. I am sorry to say, but this looks extremely suspicious.

Response: We appreciate the reviewer's concern. We acknowledge that mentioning ChatGPT in this context was inappropriate and may have caused confusion. ChatGPT was not used for any analytical or decision-making process. All data extraction, sorting, and analysis were performed manually by the authors using the exported dataset from Covidence. The reference to ChatGPT has been removed from the manuscript to ensure clarity and transparency.

Comment 19: You name a "qualitative analysis of the results was conducted". What do you mean by this, the Rob assessment? If yes, this is unnecessary here, since you have your own paragraph. And if it is the Rob2 assessment, you are not doing it because of the heterogeneity of the included papers. Theoretically, by now, you should have written the methods, but you haven't run the research.

Response: We thank the reviewer for this important comment. We clarify that the qualitative analysis refers to a narrative synthesis of the findings across studies and is independent of the risk of bias (RoB2) assessment. The RoB2 assessment was performed separately and is reported in its dedicated section. We agree that its mention in the Data Synthesis section was inappropriate and have therefore removed it to improve clarity.

Comment 20: Rob: While you named the domains assessed, which is not really mandatory, you have not provided any reference for this.

Response: We thank the reviewer for this observation. We have now added the appropriate reference for the Risk of Bias 2 (RoB2) tool in the revised manuscript.

Results

Comment 21: PRISMA chart: Figure 1 (PRISMA chart): First of all, the numbers in the first box don't fit.

Response: We thank the reviewer for this observation. We identified the inconsistency in the PRISMA flow diagram and have now corrected the numbers to ensure full accuracy and consistency throughout the figure.

Comment 22: Moreover, the left box is for the databases when you add papers from other sources; therefore, it is the right box, first row, which you left unused.

Response: We appreciate the reviewer's comment. We have revised the PRISMA flow diagram to correctly allocate records from databases and other sources in the appropriate boxes, in accordance with PRISMA guidelines.

Comment 23: Additionally, you included 2 papers from Zotero and 4 unspecified; where do they come from, and why haven't you mentioned them in the method section?

Response: We thank the reviewer for this important point. We have clarified that all records were initially managed through Zotero for reference organization and duplication. The previously labeled "unspecified" records have now been explicitly identified and their origin has been clearly described in the Methods section. The PRISMA figure has been updated accordingly.

Comment 24: And generally, when you have unused boxes or sentences, remove them.

Response: We agree with the reviewer and have simplified the PRISMA flow diagram by removing unused elements to improve clarity and adherence to PRISMA reporting standards.

Comment 25: Table 1: First, it's ok to provide a comprehensive table with key features of the included papers, but what about all the extracted information you have? Why not provide it in a supplementary table? There are some missing things: Esposito mentions BMI without any value, Georgoulis mentions AHI, but I cannot find what this abbreviation means. What is the time point? You mentioned before that you extracted the follow-up time.

Response: We thank the reviewer for this suggestion. We have expanded Table 1 and added a supplementary table (Table S1) including detailed extracted variables. We have also clarified missing abbreviations (e.g., AHI, defined as Apnea–Hypopnea Index) and added follow-up time points for each study to improve transparency and comparability.

Comment 26: Primary outcome inclusion criteria: Why have you included papers which have not mentioned the primary outcome, but just secondaries? That's flat wrong. Every paper included needs to have the primary outcome. Secondary outcomes are just bonuses. Either exclude the ones with missing primary or change the method. Based on the RQ you provided, biomarkers are a primary outcome too and not a secondary.

Response: We appreciate the reviewer's comment. As this is a scoping review, our objective was to map the available evidence rather than quantitatively synthesize effects. Therefore, we included studies reporting relevant outcomes (gut microbiota, inflammatory markers, or metabolic parameters), even when these were not defined as primary outcomes in the original studies. We have clarified this rationale in the Methods section.

Comment 27: Population: You summarised the number of participants in all the studies; this is not approximately, it's exact, in mathematics. However, this is not totally true, as the 20 from Haro 2016 are probably included in the 106 from Haro 2017. That's why it is commonly used to include the later paper with the higher number (if it's a follow-up) or focus on the most prominent paper for your review.

Response: We thank the reviewer for this observation. We have clarified that Haro et al. (2016) and Haro et al. (2017) are independent analyses within the CORDIOPREV project with different populations and follow-up periods. We have revised the manuscript to avoid double counting and ensure accurate reporting of participant numbers.

Comment 28: You mention many characteristics but do not provide detailed information. What is the sex distribution? What are the mean or median ages? You only included minimal information in Table 1, but this is insufficient.

Response: We appreciate the reviewer's comment. We have now added more detailed demographic information, including sex distribution and mean age where available, both in Table 1 and in the Supplementary Material.

Comment 29: You have mentioned the BMI ranging from ≥24, but your inclusion criteria is ≥25.

Response: We thank the reviewer for this observation. We have corrected the manuscript to ensure consistency between inclusion criteria (BMI ≥25) and reported study characteristics.

Comment 30: The sentence about the comorbidities is difficult to understand. Create two sentences: one with no comorbidities and one with comorbidities.

Response: We thank the reviewer for this suggestion. We have revised the sentence to clearly distinguish participants without comorbidities from those with cardiometabolic conditions, improving readability.

Comment 31: Intervention: "daily caloric intake ...": values? Which study had which delivery modus? Which doses, which administration (is there another administration instead of eating?)?

Response: We appreciate the reviewer's comment. We have expanded the Intervention section to include specific details on energy intake (when reported), dietary prescriptions, and intervention delivery methods across studies to improve methodological clarity.

> **Comment 32:** Outcomes: To what domains do you refer? The rob? And who do these domains and timings differ? You name three trials (don't need to talk about Haro et al. again), and referenced Buscemi, but the Table 1 says it's not them but Hernando-Rodondo et al. You say three describe biomarkers ... but you have not referenced them. You observed differences if they have been primary or secondary in the respective study, but how (and is it actually relevant at all?)?

Response: We thank the reviewer for this observation. We have clarified that outcome domains refer to gut microbiota composition, inflammatory biomarkers, and metabolic/anthropometric outcomes. We have also harmonized reporting across studies and corrected inconsistencies between Table 1 and the text.

> **Comment 33:** Primary outcome: Now you name Haro 2016 and Haro 2017 own trials, but don't provide any information on the fourth trial anymore. You mentioned in the methods qPCR or 16s rRNA, where are the data? What about the ratio you mentioned in the methods, data? Later, you mention as a limitation that values are often just available in figures, but some of the papers reported α-diversity, for instance, which could be assessed. Especially when it is known that higher α-diversity can be associated with lower inflammation (doi.org/10.1111/imr.12567). And in the end, you name Hernando-Rodondo et al as one of the papers just assessing your secondary outcome. Still, the Table says differently; in fact, it contradicts this, stating there is no information on any other outcome than the gut microbiome.

Response: We thank the reviewer for this detailed comment. We have revised the Results section to ensure consistent reporting of all microbiome-related outcomes, including α-diversity, where available. We acknowledge that some studies reported data only in figures, which limited extraction of precise numerical values. The manuscript has been corrected to resolve inconsistencies between text and Table 1 and to ensure alignment of reported outcomes across studies.

> **Comment 34:** Inflammatory markers: Sometimes I miss the p-values or at least the information on the significance at all. What is this diet-based lifestyle program? It was never mentioned before. In the paragraph about Georgoulis et al., you mention the MD and lifestyle intervention. This sounds like it is in the same intervention group. However, your inclusion criteria explicitly say that the only intervention is MD, nothing else? Does this paper then fit at all?

Response: We thank the reviewer for this important observation. We have added p-values or significance information where available. Regarding Georgoulis et al., we clarify that the intervention included a Mediterranean diet combined with structured lifestyle components (physical activity, sleep, and sedentary behavior targets) in one arm. We acknowledge that this introduces a multi-component intervention, and this has been discussed as a potential limitation in the manuscript.

> **Comment 35:** BMI & weight: Where does the MIMOSA trial come from? It was never mentioned before.

Response: We thank the reviewer for this observation. We have clarified the origin and details of the MIMOSA trial in the revised manuscript and ensured consistent reporting across all included studies.

> **Comment 36:** ROB: I am missing more precise information, especially regarding Haro et al. 2017 and the high-risk domains. I can quickly see the domain assessment in the figure, but I don't know, for example, what the problem was in the outcome.

Response: We appreciate the reviewer's comment. We have expanded the Risk of Bias section to provide more detailed explanations of domain-level concerns, particularly for Haro et al. (2017), including issues related to study design, blinding, and outcome reporting. These clarifications improve interpretability of the risk assessment.

Discussion

Comment 37: I'm sorry to say, but this is not a proper discussion. It simply repeats the results. There is no reference to other papers, either in favour of or against your findings. There is also no context for your findings within the broader research on MD and the microbiome.

Response: We thank the reviewer for their insightful comments and constructive feedback, which have been extremely valuable in improving the quality and clarity of our manuscript. In response: We acknowledge that the initial version of the Discussion was overly descriptive. To address this, we revised the section to include a deeper interpretation of our findings within the context of existing literature.

Comment 38: The paragraph on the final limitation: you aimed for a scoping review, obviously you are not doing a meta-analysis, regardless of the findings.

Response: We revised the final limitation paragraph to clarify that the absence of a meta-analysis was a methodological choice inherent to the scoping review design, not a result-driven limitation. The new text reflects that our purpose was to map available evidence rather than synthesize quantitative estimates.

Comment 39: You mentioned in the highlight of the review that MD is feasible and safe. While true, this was not a finding of your paper.

Response: The statement suggesting that the Mediterranean diet is "feasible and safe" has been removed, as these characteristics were not assessed in our included studies.

Comment 40: Conclusion. The conclusion is too long and needs to be more concise, focusing on the key findings of your research.

Response: The Conclusion section has been rewritten to be more focused and succinct, summarizing only the key findings and implications for future research. We believe these revisions substantially improve the interpretive depth, contextual relevance, and overall scientific rigor of the Discussion and Conclusion sections.

Reviewer 3

Comment 1: Congratulations for your great work, I think your question is very attractive and innovative. The method's description was well established as well as your conclusion from the systematic review you performed. One thing that get me distracted while reading was the abbreviation of Mediterranean diet, in some paragraph you used in the hole without the (MD), only mention the whole Mediterranean diet and the (MD) once and after that you can use your abbreviation in the rest of the text, also the MD is quite distracted because does recall to the M.D medical degree so if possible adjust it like Mediterranean diet (Md) maybe, if no it is okay just make sure to use it in the text instead of the Mediterranean diet once you mentioned the meaning.

Response: We appreciate your positive feedback and your helpful comment regarding the abbreviation. We have adopted "MedDiet" for Mediterranean diet throughout the manuscript, as this usage is increasingly common in the literature and reduces potential ambiguity. We now define it at first mention as "Mediterranean diet (MedDiet)" and apply it consistently across the text, tables, figures, and Supplementary Materials. All prior instances of "MD" have been updated to MedDiet.

Reviewer 4

Introduction

Comment 1: The introduction is well written and very clear. But you may consider defining the Mediterranean diet earlier in the introduction, when it is first mentioned.

Response: Thank you for this helpful suggestion. We have moved the definition of the Mediterranean diet to appear earlier in the introduction (second paragraph). This change was beneficial in providing context earlier for readers, enhancing comprehension and flow.

Methods

Comment 2: MeSH terms: In the methods, you used multiple overlapping MeSH terms. As MeSH terms are used as umbrella terms gathering multiple wording variants, using multiple similar MeSH terms is usually not needed. This is just a comment, as this does not need to be changed at this point.

Response: We acknowledge the reviewer's comment. We agree that MeSH terms act as umbrella terms that can capture multiple wording variants, and that using multiple similar MeSH terms is generally not necessary. However, we believe this approach did not compromise the comprehensiveness of the search strategy.

Comment 3: Database selection: Why was the search limited to these 3 databases? Typically, Web of Science is also included. You may also consider Cochrane.

Response: We thank the reviewer for this suggestion. We selected PubMed, Embase, and Scopus because they are the most comprehensive databases for biomedical literature relevant to our research question. Additional databases such as Web of Science and Cochrane were considered; however, they largely overlapped with the results already retrieved from the selected databases.

Comment 4: RCT justification: Even though it may be guessed from the introduction, you may consider clearly explaining why you chose to restrict your review to RCT (either in the introduction or in the methods). I guess that you wanted to assess the "pure" effect of the Mediterranean diet, without confounding by dietary supplements and other health-related behaviors.

Response: We appreciate the reviewer's comment. Our initial aim was to include only randomized controlled trials to assess the specific effects of the Mediterranean diet while minimizing confounding factors. However, due to the limited number of eligible RCTs, we expanded the scope of the review to a scoping review in order to comprehensively map the available evidence on gut microbiota, inflammatory, and metabolic outcomes.

Comment 5: PICOS redundancy: It does not seem necessary to repeat the PICOS after having defined the inclusion criteria in the text. This is a bit redundant. You may use either the text or the PICOS.

Response: We thank the reviewer for this observation. We agree that the PICOS framework is redundant when the inclusion criteria are fully described in the text. We have therefore streamlined the Methods section to avoid repetition and improve clarity.

Comment 6: Covidence citation: When mentioning Covidence, you should fully describe it in the text (or as a reference), for example as suggested here: https://support.covidence.org/help/how-can-i-cite-covidence. Of note, this type of description is standard for all devices and software described in the manuscript.

Response: We thank the reviewer for this important suggestion. We have now fully described Covidence in the Methods section and included the appropriate citation in the References, in accordance with standard reporting guidelines for software used in systematic reviews.

Comment 7: Full-text assessment: How many reviewers assessed the full texts? Also two?

Response: We confirm that full-text articles were independently assessed by two reviewers, following the same procedure as title and abstract screening. Any disagreements were resolved through discussion and, when necessary, consultation with a third reviewer. This has been clarified in the revised Methods section.

Comment 8: You may consider explaining that after the screening based on title and abstract, selected records underwent full-text assessment (instead of simply "subsequently", and you can of course explain it differently). This is obvious for those who have done such reviews, but the transition between title/abstract and full text may not be clear for each reader.

Response: We have revised the Methods section to explicitly state that after independent screening of titles and abstracts by two reviewers, potentially eligible studies were retrieved for full-text assessment based on predefined inclusion and exclusion criteria. This clarification improves transparency of the study selection process.

Comment 9: Abbreviations: In the data extraction, you should describe the new abbreviations at first use. By the way, abbreviations used in the abstract are typically explained again when they appear in the text for the first time.

Response: We thank the reviewer for this observation. We have revised the manuscript to ensure that all abbreviations are defined at their first occurrence in the main text, including C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and body mass index (BMI).

Comment 10: ChatGPT use: The use of ChatGPT is unclear in the text. Please describe it more clearly. Why was it needed?

Response: We thank the reviewer for this comment. ChatGPT was used solely as a language editing tool to assist in improving clarity and readability of the manuscript. It was not used for data analysis, interpretation, or decision-making. All scientific content was independently developed and verified by the authors.

Comment 11: Scoping vs. systematic review: When considering your methods, I actually wonder whether your work is really a scoping review. With your clear inclusion criteria and systematic approach, it looks like a systematic review. What makes it a scoping review?

Response: We thank the reviewer for this important observation. Although the initial objective was to conduct a systematic review of RCTs, the limited availability of eligible studies led us to broaden the scope. As a result, we adopted a scoping review methodology to comprehensively map the available evidence on the effects of the Mediterranean diet on gut microbiota and related outcomes, and to identify existing research gaps. This approach is more appropriate given the heterogeneity and limited number of RCTs in this field.

Results

Comment 12: Flowchart: In the results, the flowchart seems incomplete. Some numbers are missing, particularly references from other sources and other reasons. It is OK if it is 0, but then you should mention it. To improve the readability, you may want to clarify on the flowchart which step is based on title/abstract and which step is based on full text.

Response: We identified the error in the PRISMA flowchart and have now corrected it. Regarding Table 1, we have enhanced it with the collected data and believe it now provides clearer documentation of the

characteristics and outcomes of the included studies. For mean and median values, we will provide a dedicated supplementary file to present these results in greater detail.

> **Comment 13:** Data presentation: According to your methods, you collected a lot of data on your studies. But all of this does not appear in the table. It is OK not to show everything, but it seems that there is a lot of data not being shown. You may consider expanding the table, or adding tables (either as part of the article or as appendix).

Response: We appreciate this helpful suggestion. We have revised the Risk of bias assessment section to describe in greater detail the specific sources of bias identified in the study rated as high risk (Haro et al., 2017). The revised text now specifies that the open-label design, limited adherence monitoring, and unclear blinding of outcome assessors contributed to high risk in three domains (deviations from intended interventions, measurement of the outcome, and selection of the reported result). We also added a short explanation of how these biases could affect interpretation, namely, that they may have exaggerated the observed intervention effects. This revision clarifies the basis for the "high risk" rating and its implications for the strength of the evidence.

> **Comment 14:** Sample size and participant characteristics: Is the total size of the groups really approximative? It should be possible to calculate exact numbers. You may also mention the total sample size. When comparing studies, you may consider not only thresholds but also mean/median values for each of the key characteristics, as they describe the participants included. Such values may be displayed in a table. This also pertains to the daily caloric intake, which may be described more clearly. Of note, you could calculate kcal/day from kcal/kg/day by multiplying it by the average weight in kg (or the contrary).

Response: We identified the error in the PRISMA flowchart and have now corrected it. Regarding Table 1, we have enhanced it with the collected data and believe it now provides clearer documentation of the characteristics and outcomes of the included studies. For mean and median values, we will provide a dedicated supplementary file to present these results in greater detail. With respect to caloric intake, not all studies reported this information comprehensively, particularly Haro et al., 2016 and 2017, which prevents a uniform description in the main text. Therefore, we will present this variable only for studies that reported it, clearly indicated in the supplement.

> **Comment 15:** Gut microbiota as primary outcome: In fact, most included studies did not assess gut microbiota, although this is the title and primary outcome of the review. This is a problem. You may consider excluding studies without assessment of gut microbiota or explicitly refocusing your review on the effect of Mediterranean diet on gut microbiota, inflammation markers and metabolic outcomes. The lack of gut microbiota assessment in most studies limits your ability to assess whether microbiota are mediators of inflammation and metabolic outcomes, as presented in the introduction. This remains a convincing hypothesis, but you may consider framing the introduction to the answers that can be provided based on the review.

Response: We acknowledge that gut microbiota was assessed in 4 of the 8 included studies. Nevertheless, as per our pre-specified inclusion criteria, the remaining studies were retained because they addressed our secondary outcomes of interest (inflammatory markers and body composition), thereby contributing to the understanding of the Mediterranean diet → inflammation/metabolism pathway. We have added this rationale to the Methods and Discussion to reinforce the coherence of the review's scope. We appreciate your thoughtful comment about our introduction. We agree that the introduction should clearly align with what the review can answer. However, we believe that the current version already reflects this scope. The introduction explicitly states that our scoping review aims to "systematically map available RCTs, clarifying the effects of the Mediterranean diet on gut microbiota composition, systemic inflammatory biomarkers, and metabolic outcomes in adults with overweight and obesity." This sentence already defines

the main objective of the review and appropriately frames the type of answers that can be provided based on the available evidence. For this reason, we have chosen to maintain the current wording.

Comment 16: Results table: In addition to key participant characteristics, you may consider presenting key study results in a table.

Response: We expanded the Discussion to better position our findings within the broader literature on the Mediterranean diet, gut microbiota, and cardiometabolic outcomes. We now emphasize that our results align with previous evidence showing that Mediterranean-style dietary patterns promote enrichment of SCFA-producing taxa and lower systemic inflammation, thereby supporting metabolic improvement pathways mediated by the microbiota (e.g., Garcia-Mantrana et al., 2018). These updates provide a more comprehensive contextualization of how the included trials contribute to the current understanding of diet–microbiota–inflammation interactions in obesity.

Comment 17: Risk of bias: When presenting the risk of bias, it would be nice to describe more specifically the biases that were identified, particularly for the study with high risk of bias. Bias categories are visible on the figure anyway. How do these biases affect your interpretation of the findings?

Response: We have revised the Limitations section to make it more concise and avoid repetition of points already discussed earlier in the manuscript. The revised paragraph now provides a high-level summary focused on the main methodological constraints, such as the difficulty of identifying trials that simultaneously assessed the Mediterranean diet, gut microbiota, and obesity, as well as population heterogeneity and variability in follow-up duration and comparators, while omitting details already addressed in the Discussion. This restructuring improves readability and clarity while maintaining the essential contextual information.

Discussion

Comment 18: The discussion is clearly written but essentially summarizes the findings. It would be important to include a broader comparison to the literature, for example by positioning your findings within what is generally known about the Mediterranean diet and the microbiome in relation to cardiovascular/metabolic outcomes and inflammation.

Response: We appreciate the reviewer's comment. To clarify, we did perform a formal quality appraisal using the ROB 2 tool, which is now clearly described in the Methods and Results sections. Regarding meta-analysis, the main limitation is not the number of studies, but rather the substantial heterogeneity in methods, outcome metrics, and follow-up durations across trials, which precludes meaningful data pooling. Therefore, we focused on a descriptive synthesis to highlight cross-study patterns and consistency instead of effect sizes.

Comment 19: Limitations: The limitations are somewhat repetitive of points raised earlier in the manuscript. You may be more superficial in the limitations if details were explained previously or be superficial earlier in the text and more detailed in the limitations.

Response: We thank the reviewer for this comment. We have revised the Limitations section to reduce redundancy and provide a more concise summary focused on the main methodological limitations.

Comment 20: Quality appraisal and meta-analysis: The comment about a formal quality appraisal and a meta-analysis not being possible is surprising. You actually performed a formal quality appraisal using ROB2. Regarding meta-analysis, I think that the main obstacle is the heterogeneity of methods and outcome metrics across studies, which precludes data pooling, rather than the number of studies.

Response: We appreciate the reviewer's comment. We have expanded the Risk of Bias section to provide more detailed explanations of domain-level concerns, particularly for Haro et al. (2017), including issues

related to study design, blinding, and outcome reporting. These clarifications improve interpretability of the risk assessment.

Comment 21: Implications: The statement that this review highlights important implications for research and practice is encouraging, and you may therefore want to describe these important implications in a more assertive manner.

Response: We thank the reviewer for this suggestion. We have revised the Conclusions section to present the implications for research and practice in a more clear and assertive manner.

References

Comment 22: Layout and references: And you should consider homogenizing the layout (line spacing, break to next line), the citation reference format (e.g., using Zotero, Mendeley or Endnote) and the numbering of the affiliations.

Response: We thank the reviewer for this comment. We have homogenized the manuscript layout, standardized the citation format using APA style using Zotero, and corrected the numbering of affiliations to ensure consistency.

Comment 23: Final note: Finally, I want to emphasize again that, despite my critiques, this manuscript remains a fabulous work. I congratulate you again!

Response: We sincerely thank the reviewer for the positive feedback and encouraging remarks. We greatly appreciate the time and effort dedicated to reviewing our manuscript and the valuable suggestions provided, which have helped improve the quality of the work.