Peer-Review comments and authors responses

"Digestive Supplement Use and Its Association with Abdominal Pain in the Past 12 Months: Findings from the U.S. NHANES 2017–2018 Survey."

Dear Dr. Fregni

We sincerely appreciate your detailed review and constructive comments on the manuscript "Digestive Supplement Use and Its Association with Abdominal Pain in the Past 12 Months: Findings from the U.S. NHANES 2017–2018 Survey." We appreciate you and the reviewers for your time, attention, and valuable observations, which contributed significantly to the quality and clarity of the manuscript. We have carefully analyzed each comment in detail with your relevant revisions.

Throughout this letter, we provide detailed responses to each comment, along with a specification of all revisions made to the manuscript. In addition, a copy of the track changes document and the clean copy of the manuscript with the changes implemented and reflected are attached.

Reviewer 1

Esteemed Reviewer 1, we sincerely appreciate your thoughtful feedback, which has been invaluable in refining our analytical model and guiding the approach adopted for the revision of the manuscript. Below, we provide a detailed and structured response to each of your comments.

> **Comment 1:** Clinically relevant and increasingly common issue. The use of a large and nationally representative dataset (NHANES) strengthens its external validity. The paper is generally well structured, with a logically developed narrative, and appropriate statistical methods.

Response: We sincerely thank the reviewer for the positive feedback and encouraging comments. We greatly appreciate the recognition of the study's relevance, structure, and methodological approach.

Major Concerns

<u>Methods</u>

> **Comment 2:** Cross-sectional design + reverse causality not sufficiently addressed. The exposure (supplement use) is likely a response to the outcome (abdominal pain), not a cause. While the authors acknowledge this in passing, this issue should be central to discussion and conclusion. In this case it is highly recommended to perform sensitivity analyses excluding individuals with chronic illness or explicitly restrict conclusions to association only with stronger cautionary language.

Response: Thank you for this valuable comment. We fully agree that addressing potential reverse causality and evaluating robustness through sensitivity analyses would strengthen the findings. Although NHANES does not allow exclusion of all individuals with chronic conditions due to data availability and sample size constraints, we incorporated multiple potential confounders and comorbid factors (e.g., age, BMI, diabetes, anxiety, sleep disturbances) in our weighted multivariable model. This approach serves as a form of internal sensitivity analysis, assessing whether the association between supplement use and abdominal pain persisted after controlling for relevant health and behavioral variables. We also revised the Discussion and Conclusion to emphasize that our findings represent associations only and should not be interpreted as causal.

> **Comment 3:** Exposure definition is oversimplified and under-validated. "Supplements to improve digestion" is treated as a single binary variable. From NHANES, this item includes diverse

categories (probiotics, botanicals, digestive enzymes, etc.), many with opposite mechanisms. Recommendation: Provide descriptive breakdown by supplement subtype, dosage, or duration — or explicitly state these limitations earlier.

Response: We appreciate the reviewer's insightful comment. Unfortunately, the NHANES dataset does not provide detailed information on supplement subtype, dosage, or duration. We have now acknowledged this limitation in the revised manuscript and noted that future studies should aim to collect more granular data on specific supplement categories.

Comment 4: Confounding structure not adequately justified. Only four covariates were included (age, sex, BMI, diabetes), claiming "strongest evidence" — but GI conditions, medication use, anxiety, diet, smoking, alcohol, SES are strong known confounders. The DAG presented is over-simplified relative to the literature. Recommendation: Expand confounder rationale and perform sensitivity models including available variables (education, income, smoking, etc.).

Response: Thank you for this important observation. We agree that the initial confounder selection was limited and that additional variables—such as socioeconomic, behavioral, and psychosocial factors—could influence the relationship between supplement use and abdominal pain. In the revised analyses, we expanded the confounder set to include education level, income-to-poverty ratio (PIR), smoking status, alcohol consumption, anxiety, sleep disturbance, and history of gallbladder surgery, in addition to age, sex, BMI, and diabetes. Each potential confounder was assessed individually using survey-weighted logistic regression models to evaluate its independent association with the outcome and its effect on the main exposure estimate. Variables that altered the supplement–abdominal pain association or showed theoretical relevance were retained in the final multivariable model. This expanded modeling strategy, combined with the application of survey-weighted methods to account for the NHANES complex sampling design, strengthens the validity and generalizability of our findings. While residual confounding due to unmeasured variables (e.g., medication use or dietary composition) cannot be entirely excluded, our final model incorporates the most relevant covariates available within the dataset.

<u>Results</u>

Comment 5: Statistical inconsistencies and unclear modeling decisions. Both t-tests and rank-sum tests are reported; primary test decision seems arbitrary. Very low pseudo-R^2 (0.0132) suggests insufficient explanatory power — this deserves interpretation. Effect modification explored without clear clinical justification for all selected modifiers. Recommendation: Strengthen statistical rationale, especially for interaction terms.

Response: We appreciate the reviewer's valuable feedback on the statistical interpretation. We have carefully reviewed the statistical rationale and clarified the justification for the predefined effect modifiers in the Statistical section. Both parametric (t-test) and nonparametric (Wilcoxon rank-sum) tests were applied to ensure robustness, given the non-normal distribution of continuous variables but homogeneity of variances. All analyses incorporated NHANES survey weights, primary sampling units, and strata using the svy design commands, to account for the complex, multistage sampling structure and to generate nationally representative estimates. The low pseudo-R^2 (0.013) observed in weighted logistic models is consistent with cross-sectional analyses of population-based data, where outcomes are multifactorial and design-based variance estimation often attenuates model fit statistics. Therefore, the model's adequacy was judged based on significance of predictors and overall Wald test results rather than pseudo-R^2 magnitude.

Minor Concerns

<u>Introduction</u>

Comment 8: Introduction overly long and repetitive. Streamline paragraphs 2–3

Response: We appreciate this helpful suggestion. The Introduction has been revised to eliminate redundancies and improve readability. Specifically, we condensed paragraphs 2 and 3, combining overlapping content on the definition, regulation, and effectiveness of digestive supplements. The revised version maintains a clear logical progression: (1) contextualizing dietary supplement use, (2) defining digestive supplements and their public health relevance, and (3) summarizing evidence gaps that justify the present analysis. This restructuring shortens the section while improving clarity and focus, in line with the reviewer's recommendation.

Comment 6: Figures lack confidence intervals and key sample size information. Add error bars, more readable axes.

Response: We thank the reviewer for this helpful suggestion. Confidence intervals and sample size information have now been added to the figures, and the axes have been revised to improve readability.

Comment 7: Table 3 & 4 headings unclear regarding reference groups. Please clarify the direction of effects.

Response: We thank the reviewer for this helpful comment. We have clarified the direction of effects by adding notes below each table, and we have revised the titles of Tables 3 and 4 to explicitly indicate the reference group for each categorical variable.

Reviewer 2

Dear Reviewer, 2, the observations regarding reverse causation, cross-sectional design limitations, and the importance of addressing missing data have been especially valuable in refining the methodological clarity and interpretative caution of our work. We greatly appreciate your thoughtful and insightful review.

Major revisions

<u>Methods</u>

Comment 1: A major issue in your study is reverse causation, since this is cross-sectional data and there is solid biological plausibility for the reverse association (abdominal pain leading people to consume certain supplements). Although acknowledged that this is a limitation of your data that cannot be solved, your Discussion section should bring this issue more extensively in the limitations section. This is an exploratory, hypothesis-generating study, rather than hypothesis-testing. Even though you mention that the cross-sectional design limits causal inference, this should be expanded and the issue of reverse causation should be brought up.

Response: We appreciate the reviewer's thoughtful observation. We fully agree that the cross-sectional nature of NHANES limits causal inference and that potential reverse causation must be explicitly addressed. In the revised Discussion, we have expanded this limitation and clearly stated that the present work represents an exploratory, hypothesis-generating study rather than a hypothesis-testing design. We now emphasize that the directionality of the observed association cannot be determined and that individuals with preexisting gastrointestinal symptoms may have been more likely to initiate supplement use rather than supplements leading to pain onset. This clarification strengthens interpretive caution and aligns with the study's exploratory scope. In addition, the analytic strategy now includes a broader set of potential confounders (education, income, smoking, alcohol use, anxiety, sleep disturbance, and gallbladder surgery), which improves the robustness of the association estimates and partially mitigates, but does not eliminate, the limitations inherent to the cross-sectional design. We have also clarified this rationale in the Methods and Discussion sections to better reflect the analytical depth of the revised manuscript.

Minor revisions

> **Comment 2:** Under Methods > Variables: Avoid the use of bullet points in these subheaders (outcome, exposure, covariates, effect modifiers). Alternatively, each of these bullet points can be a paragraph.

Response: You have made some important consideration regarding the writing fluency of this paper. We have, accordingly, adjusted our methods section to concise paragraphs, improving the original bullet points format.

Discussion

> **Comment 3:** Under Discussion: You should also mention the issue of missing data in your limitations, since you only used complete cases in your analysis and lost a big part of your sample size because of that. Although this is an acceptable approach, this should be discussed as a limitation, since this can introduce bias.

Response: We thank the reviewer for this valuable observation. We agree that the use of complete-case analysis may have introduced bias due to missing data and the resulting reduction in sample size. We have now acknowledged this issue in the discussion with the following text: "Our analyses were restricted to complete cases, which led to the exclusion of a substantial proportion of the original NHANES sample. Although this is a statistically acceptable approach, we recognize that it may have introduced bias and reduced the representativeness of the results. This reduction in sample size could have affected the precision and generalizability of our estimates. Future studies should consider using multiple imputation or other missing-data methods to minimize possible distortions." This addition directly addresses the reviewer's concern and transparently acknowledges the methodological trade-offs.

Reviewer 3

Dear Reviewer 3, We sincerely appreciate and highly value your thoughtful feedback, which has been essential to improving the overall clarity and consistency of our manuscript. In accordance with your suggestions, we have refined the manuscript formatting and clarified the rationale of the analysis.

Methods

> **Comment 1:** The work is well-structured and clearly follows its proposal. However, the authors should mention in the justification why they selected a population of adults over 20 years of age and clearly state the inclusion and exclusion criteria.

Response: We thank the reviewer for the positive feedback and appreciation of the manuscript's structure and clarity. We have expanded the justification for selecting participants aged 20 years and older and have now clearly stated the inclusion and exclusion criteria in the Methods section of the revised manuscript.

> **Comment 2:** The authors are encouraged to improve the quality of the images.

Response: We thank you for this valuable suggestion. As recommended, the quality of all images has been improved. Images were reformatted and recreated directly within the manuscript to ensure better visual integration.

Reviewer 4

Dear Reviewer 4, We deeply appreciate your comprehensive and insightful review. Your comments have been of great value in refining the conceptual framework, improving methodological justification, and strengthening the scientific relevance of our manuscript.

Methods

Comment 1: About the justification for the data-source and academic context: The manuscript would benefit from a more robust rationale for using the NHANES dataset. While I understand this work originated from an academic exercise, the published paper -if accepted- must be justified on its own scientific merits. The current justification is lacking. I recommend you and the co-authors explicitly acknowledge the hypothesis-generating nature of NHANES and build a more stronger, positive argument for how its specific data-structure, despite not being designed for this specific goal, is nevertheless a valid and powerful tool to address the research question. This may transform a perceived limitation into a demonstration of methodological sophistication. Moreover, the statement "This dataset was provided as part of the PPCR course for educational purposes" in the Methods section undermines the manuscript's standing as an independent scientific contribution. I strongly recommend moving this acknowledgment to the acknowledgements section at the end and rephrasing it, for example: "Authors thank the HMS-PPCR for facilitating access to the NHANES dataset." The methods section should focus solely on the scientific logic and rationale for the data source. Consider discussing these fundamental limitations in the discussion section. The conclusions must be carefully framed to acknowledge that the results reflect a general association with a non-specific outcome and a broadly defined exposure, preventing more nuanced causal or mechanistic inferences.

Response: Thank you for pointing this out. We have revised the Study Design and Data Source section to provide a stronger scientific rationale for using NHANES as our data source. Specifically, we emphasize the survey's methodological strengths, its nationally representative sampling design, standardized data collection procedures, and comprehensive health-related domains, which make it uniquely suited for hypothesis-generating population-based research. We also reframed the description to highlight NHANES as a valid and powerful tool for examining associations between health behaviors and clinical outcomes, despite its cross-sectional nature. In accordance with the reviewer's recommendation, we have removed the reference to the dataset being provided for educational purposes from the Methods section. This acknowledgment has been relocated and rephrased in the Acknowledgments section as follows.

Comment 2: More focus for the research narrative: The introduction establishes a specific focus on "probiotics," but the methodology employs a much broader exposure variable ("digestive supplements"). This creates a disconnect between the posed research problem and the actual analysis. I think it is possible to tighten the narrative by up to two options: a) By reframing the introduction to consistently focus on the broader category of "digestive supplements" from the outset, or b) By providing a clear justification in the methods for why the broader category is a valid proxy for investigating probiotics specifically.

Response: We thank the reviewer for this insightful comment. We agree that the initial framing emphasized probiotics too narrowly compared with the broader exposure variable analyzed ("digestive supplements"). In the revised manuscript, we have realigned the narrative throughout the Introduction to focus consistently on the broader category of digestive supplements, explicitly noting that probiotics are one of several subtypes within this group. This adjustment ensures conceptual coherence between the research rationale and the analytic definition used in NHANES. We also added a sentence in the Methods to justify this operational choice: "Digestive supplements were defined as any self-reported supplement used to improve digestion, encompassing a heterogeneous group of products (e.g., probiotics, enzymes, botanicals). This operational definition captures real-world supplement behaviors as represented in NHANES." This clarification bridges the gap between the conceptual background and the available dataset, strengthening the internal consistency of the manuscript.

Methods

Comment 3: Critical variable definitions:

> **Comment 3.1:** Outcome Variable: Combining "pain" and "discomfort" into a single binary outcome is quite problematic. These are distinct constructs with likely different underlying etiologies and clinical significance. A patient's motivation to report "discomfort" and their recall of it over a 12-month period is fundamentally different from their recall of significant "pain." This conflation, combined with the long recall period, introduces considerable potential for recall bias and measurement error, critically threatening the internal validity of the findings.

Response: We appreciate this insightful observation. We agree that the use of a single combined outcome ("pain or discomfort") introduces conceptual heterogeneity and potential measurement error. In the revised Discussion, we have added a specific paragraph acknowledging this limitation and its implications for internal validity: "The outcome variable—combining 'pain' and 'discomfort' over a 12-month recall period—captures a broad and heterogeneous construct that may be prone to recall bias and measurement error. This definition reflects a general association with non-specific gastrointestinal symptoms rather than a precise clinical diagnosis." By explicitly addressing the potential recall and construct validity issues, we acknowledge that the findings reflect an association with a non-specific symptom measure, consistent with the reviewer's recommendation.

> **Comment 3.2:** Critical variable definitions: Exposure Variable ("Took supplements to improve digestion"): This variable is overly broad and ambiguous. It does not distinguish between prophylactic use, use for mild discomfort, or use for specific pain. It also fails to capture the motivation (self-initiated vs. prescribed), specific type (e.g., probiotic, enzyme), or dosage of the supplement. This lack of specificity severely limits the interpretability of the results, as the "exposure" represents a heterogeneous group of behaviors and products. Consider discussing these fundamental limitations in the discussion section. The conclusions must be carefully framed to acknowledge that the results reflect a general association with a non-specific outcome and a broadly defined exposure, preventing more nuanced causal or mechanistic inferences.

Response: We agree with the reviewer that the exposure variable is broad and heterogeneous. The NHANES dataset does not provide detailed information on supplement subtype, dosage, motivation for use, or specific product characteristics (e.g., probiotics, enzymes, herbal products). Accordingly, this variable captures a heterogeneous set of self-reported behaviors rather than a uniform clinical exposure. In the revised Methods section, we have clarified how this variable was operationally defined in our analysis ("took supplements to improve digestion") and explicitly acknowledged its limitations in the Discussion. We now state that this exposure reflects a general behavioral pattern rather than a pharmacologically homogeneous intervention, which restricts the interpretability of the results and precludes mechanistic inference. To address this limitation, the Discussion now includes the following clarification: "The exposure variable ('took supplements to improve digestion') lacks granularity regarding supplement type, dosage, motivation, and indication, representing a diverse group of behaviors rather than a unified exposure. These issues limit interpretability and preclude mechanistic conclusions. Our findings should therefore be interpreted as reflecting a general population-level association rather than a causal effect." Furthermore, we reframed the Conclusions to emphasize that the observed association should be interpreted as exploratory and hypothesis-generating, consistent with the cross-sectional design of NHANES. We also note that future research should determine whether supplement use precedes the onset of abdominal pain or, conversely, whether preexisting symptoms motivate supplement use.