Response to editors

Title of Article: **Association Between Adequate Zinc Intake and Reduced Childhood Obesity Risk: A secondary analysis from NHANES 2017-2018**

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Dear Editor,

We thank the reviewers for their time and valuable feedback. We carefully considered their comments and made substantial revisions to address their concerns. Below, we address all comments, highlighting the changes made to improve the manuscript.

Specific Responses:

Comment 1: *I think you have to analyze your results in the multivariate analysis as a whole. I don´t think is correct for you to separate the socioeconomic analysis in the model, or the fiber intake, form the model, and analyze them separately. If you can analyze them separately, you need to perform an univariate analysis of this variables (not shown in your study now). When you perform a multivariate analysis, the relationship you find in 1 variable is affected by the other variables in the model.*

Response 1: Thank you for pointing out the need to consider multivariable analysis holistically. In the revised manuscript, we carefully interpret the findings within the multivariable framework. We emphasize the association of zinc adequacy with obesity after adjusting for socioeconomic factors, dietary intake (e.g., fiber, fat, and protein), race/ethnicity, and comorbidities. These relationships are discussed collectively in the revised Discussion section to avoid isolating individual variables without proper context. Univariable results are retained in the "Results" section as exploratory findings to complement the multivariable model.

Comment 2: *You found that kids with adequate intake of vit D had a higher rate of obesity. You didn´t discuss this result in your discussion, even though it´s one of your major results according to your title and abstract. I would suggest adding some information about this.*

Response 2: We thank you for raising up this point. Considering the limitations of our dataset regarding vitamin D (small sample size), we have removed vitamin D as a variable from the analysis and manuscript. This exclusion ensures the study's robustness and avoids potential biases. The revised manuscript now exclusively focuses on zinc intake and its association with childhood obesity.

Comment 3: *You add a figure in your submitted documents, but I couldn´t find a reference to the figure in the manuscript.*

Response 3: We appreciate this observation. All figures are now clearly referenced within the "Results" section, ensuring that their inclusion aligns with the narrative of the manuscript.

Comment 4: *Overall, you have an interesting work. Your analysis is well done, but I think there is a problem in how you are interpreting the results. You cannot separate the multivariate analysis and only talk about one variable of it separate from the others. You have to analyze them as a relationship between all the variables.*

Please find attached the files with my comments.

Response 4: Thank you again for your thoughtful comments to improve our manuscript. We carried out a careful analysis of the best presentation of the results and a careful discussion of the data in full, considering the final model.

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Reviewer #2:

1. **Title:**

Comment 1: *The title doesn't clearly state the study design. It should include mention of it with a commonly used term ("cross-sectional" might be appropriate here).*

Response 1: Thank you for highlighting this point. Considering that our study is a secondary analysis, we have now included the study design in the title. It is now titled as follows: "Association Between Adequate Zinc Intake and Reduced Childhood Obesity Risk: A secondary analysis from NHANES 2017-2018".

2. ***Abstract***

Comment 1: *The study design should also be specified in the Methods section of the abstract. In the Results, several issues need attention. First, it is unclear whether vitamin D was analyzed as a continuous variable or categorized into groups (e.g., high vs. low vitamin D). Additionally, the direction of the relationship is ambiguous; the phrase 'a statistically significant increase in the odds of childhood obesity associated with vitamin D' does not clarify whether the increased odds of obesity are associated with higher or lower vitamin D levels.*

If vitamin D was treated as a dichotomous variable, a clearer way to report the result would be: 'After adjusting for confounders, children with higher vitamin D intake had significantly greater odds of obesity compared to those with lower intake (OR = 1.91; 95% CI 1.06 to 3.42; p = 0.030).'

Alternatively, if vitamin D was considered a continuous variable, a more precise sentence could be: 'After adjusting for confounders, each 10 ng/mL increase in vitamin D levels was associated with higher odds of childhood obesity (OR = 1.91; 95% CI 1.06 to 3.42; p = 0.030).'

To enhance readability, the reporting of odds ratios should be consistent throughout, using a standardized format for each item. For instance, separate each element (OR, CI, and p-value) with semi-colons: 'OR = 1.91; 95% CI 1.06 to 3.42; p = 0.030.' Whenever possible, report the exact p-value rather than a threshold value.

In the conclusion part of the abstract, care must be taken to not overestimate the findings. Given the limitations of cross-sectional studies in establishing predictive relationships, the term "predictors" might not be appropriate here, as it implies a causal or temporal relationship. A better way to phrase this would emphasize associations rather than prediction. It would also strengthen the conclusion to acknowledge the limitations in establishing causality while emphasizing that these findings are valuable for hypothesis generation and may serve as a basis for further longitudinal research to explore these associations over time.

Response 1: Thank you for your feedback on the abstract. We want to clarify an important point on this comment. Considering that the inclusion of the vitamin D variable was introducing bias to our study secondary to a limitation of the dataset used (small sample size), the vitamin D variable was removed from the analysis. Therefore, the study focuses only on the association between zinc intake and childhood obesity. The study design is now stated on both the methods section of the abstract, and in the manuscript. Additionally, as the recommendations for reporting vitamin D are not applicable to the manuscript, we implemented them for reporting the results obtained from zinc analysis. We also made consistent the reporting of odds ratio throughout the manuscript and included the exact p-values. We use the format “OR = 0.50; 95% CI: [0.26–0.97]; p = 0.042” consistently across all sections. Finally, we replaced the word “predictors” and instead used “associations”. We thank you again for these valuable insights.

3. Introduction

Comment 3: *The scientific background and rationale for the current study are really well expressed. The knowledge gap established is that of mechanisms that link vitamin D deficiency and obesity. There is no mention of a knowledge gap that involves Zinc, which could be improved. Also, as we're dealing with a cross-sectional analysis, insights into mechanisms cannot be appreciated. Therefore, the statement of the knowledge gap must be reformulated to fit the current analysis.*

The sentence 'this study examines whether adequate dietary intake of zinc and vitamin D is associated with a reduced risk of childhood obesity' should be rephrased, as cross-sectional analyses do not estimate risk.

The statement about informing public health policies may be somewhat ambitious, as it implies that the findings could directly guide interventions aimed at reducing obesity rates and improving long-term outcomes. Since cross-sectional studies cannot establish causality, they are more suited to identifying associations rather than directly informing policy decisions. Acknowledging this limitation and framing the

findings as contributing to understanding associations that could guide future research would provide a more accurate representation.

Response 1: We want to thank you for these great insights for improving the introduction section, we appreciate all of them. Although we mention that there is a gap in the mechanisms through which zinc influences obesity, we also mention that there are gaps in the association with other risk factors, and we clearly stablish the aim of our study as follows: "this study examines whether adequate dietary intake of zinc is associated with a reduced odds of childhood obesity". Therefore, we are aiming to evidence the associations between zinc intake and childhood obesity, and not to stablish the mechanisms of this association. We also removed the word "risk" in that sentence. The revised "Introduction" section now emphasizes gaps in understanding zinc's role in obesity prevention. We have removed references to mechanisms beyond the scope of a cross-sectional study and reframed the study's contribution as hypothesis-generating rather than causal. This adjustment ensures alignment with the study design.

4. **Methods.**

Comment 1: *Study design: Although the data source (NHANES 2017-2018) is specified, it would be beneficial to explicitly label this as a "cross-sectional study" early on in the methods section. It would also be an improvement if NHANES was described briefly. Consider this quote as inspiration:*

"The NHANES is a biannual cross-sectional study conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC). The purpose of the NHANES is to collect data about the health, nutritional status, and health behaviors of the noninstitutionalized civilian resident population of the U.S. The NHANES data were obtained using a complex, multistage probability sampling design to select a sample representative of the U.S. civilian household population" [1].

Setting and Participants: The authors have clearly established that data were drawn from the NHANES 2017-2018 dataset, which provides health, nutritional, and demographic data for a representative sample of the U.S. population. However, it would be helpful to clarify that this analysis specifically used data from participants aged 2-18 years, as the current wording could be misinterpreted to suggest that NHANES only surveys this age range. Including a direct reference to the NHANES 2017-2018 target population overview (https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/overview.aspx?BeginYear=2017) would further enhance context.

Also, a more detailed rationale for the exclusion of cases with missing data is warranted. The current citation (CDC, 2022) appears to reference the consequences of obesity, which is unrelated to the treatment of missing data in this analysis

Variables and Measurements: The definition of obesity is thorough, and the authors have clearly detailed the categorization of the independent variables. However, the measurement process for these variables is not well defined. It's essential that readers understand how these variables were initially measured before categorization. Consider including information similar to the following as guidance:

'The NHANES dietary intake data were used to estimate the types and amounts of foods and beverages consumed during the 24 hours prior to the interview, as well as nutrient intake from those foods and beverages. Vitamin D, added to the nutrient list in 2007/2008, reflects the combined ergocalciferol and cholecalciferol content of foods reported by participants.' [1]

Providing similar measurement details for both vitamin D and zinc would improve the overall quality of the paper.

Bias: Discussing potential sources of bias would enhance the study's clarity and transparency. For instance, addressing known limitations in the NHANES dataset and the measurement limitations of 24-hour dietary recall would help readers understand possible biases. In addition, consider mentioning the potential influence of social desirability bias on dietary recall data.

Study Size: As this is a secondary analysis, describing how the study size was reached in detail isn't necessary. The authors describe the sample size in the results section in an adequate manner.

> *Statistical Analysis: The statistical approach is clear, but it lacks a detailed description of methods for handling missing data. This could include specifying whether missing data were assumed to be missing at random or if any sensitivity analyses were conducted to assess their impact on findings. In addition, while the manuscript addresses key aspects of logistic regression, including the binary outcome and multicollinearity checks via VIFs, it does not fully address all assumptions. Specifically, consider verifying linearity between continuous predictors and log odds of the outcome and evaluating the presence of influential outliers. Addressing these points would provide a more comprehensive assurance that logistic regression assumptions were met. Also, the VIF acronym is used without prior definition in this section.*

Response 1: We thank the reviewer for these great comments. The study design is now clearly stated early in the methods section. A brief description of NHANES has been added, including its purpose, design, and data collection processes. The mentioned citation was incorrectly placed and already removed; we thank you for noticing the issue. Explanations of body measurements, demographic and socioeconomic information, and dietary data are available on the “Data collection” section. We added a discussion of potential biases, including the limitations of 24-hour dietary recall and missing data in the “Limitations” section of the manuscript. The manuscript does not have now the description of the study size in the methods section. The assumption of "missing at random" is explicitly stated, and the potential impact of missing data on findings is acknowledged in the “Discussion” section. The VIF acronym is now stated in the “Covariates” section.

5. Results.

Comment 1: *Participants and Sample Characteristics: The results section provides useful information on sample demographics and obesity prevalence. However, it would be helpful to specify the number of children with complete data for each variable to clarify the impact of missing data. One way to achieve this is by including the 'n' for each variable in Table 1 (e.g., Dietary Fiber (gm), n = x). This approach allows readers to compare each 'n' with the total sample size to gauge the proportion of missing data for each variable.*

Descriptive Data and Outcome Events: The description of variables like vitamin D and zinc adequacy is informative, and Table 2 provides the number of children in each adequacy category within the obesity group. This inclusion adds helpful context to the observed associations.

Unadjusted and Adjusted Model Reporting: Both unadjusted and adjusted models are clearly reported, including odds ratios, confidence intervals, and p-values. The covariates included in the adjusted model are listed in the Methods section; however, to improve clarity, it would be helpful to briefly explain the rationale behind selecting these covariates as confounders. Additionally, an explanation of how continuous covariates, such as dietary fiber intake, were handled in the model would enhance understanding. Specifically, clarify whether these continuous variables were assumed to have a linear relationship with the outcome or if any transformations (e.g., categorization, polynomial terms) were applied to address potential non-linearity. This additional detail would help readers interpret how continuous variables were modeled and whether adjustments were made to meet logistic regression assumptions.

Multicollinearity Check: The use of variance inflation factors (VIFs) is noted for assessing multicollinearity. Including a brief interpretation of the VIF values would clarify why multicollinearity was considered non-problematic in this analysis.

Sensitivity Analyses: Reporting any sensitivity analyses, particularly those examining the potential impact of missing data, would enhance the robustness of the findings.

Statistical Reporting: For consistency and precision, consider reporting p-values uniformly, such as "p = 0.052" rather than "p > 0.05." This consistency would enhance readability and aid interpretation.

Response 1: We thank you for these insightful comments, they are very valuable for our manuscript. As recommended, we included the number of children with complete data for each variable in the Table 1. This information was categorized as obese and non-obese and is now available in the mentioned table as the total of participants per category, and the total amount of participants per variable per category. The rationale for including covariates is mentioned in the "Methods" section, and the role these covariates played in the model, for both the unadjusted and adjusted model is explained in the "Results" section. The interpretation of variance inflation factors (VIFs) is included to demonstrate that multicollinearity is not a concern. The reporting of p-values is now consistent throughout the manuscript, aiming to enhance readability and interpretation, as you suggested.

6. **Discussion.**

Comment 1: *Before addressing specific points, it's useful to revisit the purpose of the discussion section. This section should interpret and contextualize the study's findings in relation to the research questions, objectives, and existing literature, while also addressing limitations and potential impacts.*

"The mean age of the children in this analysis was 9.63 years, with a balanced gender distribution of 50.04% females." This descriptive detail is more appropriate for the Results section.

"The racial and ethnic diversity of this cohort, comprising 31.1% non-Hispanic white, 23.2% non-Hispanic black, 20.9% other race, 17.2% Mexican American, and 7.5% other Hispanic, highlights the role of cultural factors in health interventions." This statement is not supported by the study's analysis, as it does not evaluate the effects of interventions or provide evidence of differential impacts across these subgroups.

"Socioeconomic and cultural influences may shape dietary practices, resource access, and ultimately, obesity rates (NHANES, 2022)." While this general statement is valid, it is unclear how it directly relates to the study's specific findings, as no results here directly examine these influences on obesity rates within this sample.

Regarding Socioeconomic Status (SES): The discussion highlights a significant association between higher income and reduced obesity odds in the zinc model (OR = 0.66, 95% CI: 0.47 to 0.92, p=0.015), suggesting a protective effect. Since SES was included primarily to adjust for confounding and was not a primary variable of interest, emphasizing this finding may be misleading. Additionally, this association was not significant in the vitamin D model, which suggests that the SES finding may be incidental rather than robust. Reporting this selective result could be perceived as "cherry-picking" or a "fishing expedition," as it wasn't a primary focus of the analysis.

Regarding Dietary Fiber Intake: Similarly, the association between dietary fiber intake and increased obesity odds is emphasized, with significance noted in both models (OR = 1.14, p = 0.047; OR = 1.07, p = 0.013). Although statistically significant, dietary fiber was included as a confounder rather than a primary variable of interest, and the low odds ratios suggest limited clinical relevance. Emphasizing this finding without context could mislead readers regarding its importance.

To improve clarity and transparency, consider reframing both the SES and dietary fiber findings as exploratory results or omitting them from the main discussion unless they were prespecified as secondary objectives. This approach ensures that the discussion aligns with the study's original objectives and avoids overstating incidental findings.

Public Health Implications: While the discussion raises relevant public health concerns, the language could better reflect the limitations of cross-sectional data in directly informing interventions. Framing the results as exploratory and emphasizing that the findings suggest associations warranting further research would provide a more cautious and balanced interpretation.

External Validity: The discussion acknowledges the diversity of the cohort but could elaborate on the external validity of these findings. Comparing the sample's demographic composition to other populations, such as those in middle- or low-income countries, could provide valuable context for readers considering the broader applicability of these findings.

Study Limitations: One major limitation of the study is the disparity between the total sample size (2,704 children) and the number of participants with available vitamin D and zinc data. Vitamin D data are available for only 647 participants, and zinc data for 2,233 participants. This discrepancy significantly reduces the effective sample size for analyses involving these nutrients and may introduce selection bias if those with missing data differ systematically from those included in the analysis. This limitation should be

addressed in the discussion, with consideration of how the reduced sample size may impact the generalizability and robustness of the findings, especially for vitamin D analyses. Discussing potential reasons for the missing data and its possible effects on the results would improve transparency and rigor. In addition, given the cross-sectional nature, further longitudinal studies are necessary to confirm the associations found here and assess for possible causality.

Comparison with Existing Literature: The discussion would greatly benefit from comparing the study's findings on the associations between the primary independent variables (e.g., vitamin D and zinc intake) and childhood obesity with results from other studies that examined similar relationships. Specifically, examining whether the direction and magnitude of these associations align with previous research could provide valuable context for readers. Where discrepancies exist, the discussion could explore possible reasons, such as differences in sample demographics, data collection methods, or study design. This comparison and interpretation would add depth to the discussion, helping to position the study within the broader body of literature on micronutrient intake and obesity.

I'd like to recommend that, overall, this section be rewritten to better align with the purpose of a discussion section. Incorporating comparisons with existing literature, clearly addressing study limitations—particularly the issue of missing data—and focusing on the interpretation of the study's primary findings will strengthen the discussion's clarity and relevance. This approach will provide readers with a more balanced understanding of the study's contributions, limitations, and potential implications.

Response 1: Special thank you to the reviewer for these detailed comments on such an important section of the manuscript. The discussion section has been revised and restructured to interpret and contextualize the study's findings as suggested. The descriptive details that you mentioned regarding the mean age and gender distribution, along with the ethnic and racial distribution, and socioeconomic status of the study population were removed from the manuscript. The implications of SES and race/ethnicity in the adjusted models are discussed, with caution against overinterpreting exploratory results. As mentioned in this section, SES and race were included as confounders to control for their influence in the variable of zinc adequacy, and the relationships are interpreted as exploratory rather than independent associations. Regarding the variable of dietary fiber, after eliminating the vitamin D variable, the new model showed to be significant for this variable in the unadjusted model but non-significant in the adjusted model. Therefore, this finding is no longer emphasized in our analysis. This section has been revised to compare our findings with existing literature on zinc and childhood obesity. Studies are cited to contextualize our findings. Limitations related to the cross-sectional design, missing data, and reliance on self-reported dietary recall are emphasized. These are balanced with the study's strengths in contributing to hypothesis generation. The public health implications now stress the need for longitudinal research and cautious interpretation of findings for policy guidance.

Final Comment:

Due to the issues noted above, particularly in the discussion section, my recommendation is that this paper be revised and resubmitted for review.

References:

Orces, C., Lorenzo, C., & Guarneros, J. E. (2019). The Prevalence and Determinants of Vitamin D Inadequacy among U.S. Older Adults: National Health and Nutrition Examination Survey 2007-2014. Cureus, 11(8), e5300. https://doi.org/10.7759/cureus.5300

Response: Thank you for your thoughtful feedback, which has significantly improved the clarity, rigor, and focus of our manuscript. We are confident that the revised manuscript addresses your concerns and provides a clear, comprehensive analysis of the relationship between zinc intake and childhood obesity.