

RESPONSE TO REVIEWER'S COMMENTS to MANUSCRIPT NO. 88545

REVIEWER	Our response	Location of changes in manuscript
Reviewer 1: “...the study did not analyze molecular biomarkers”	We revised the paragraph discussing biomarkers and moved it to the discussion section beginning on line 664 and ending on line 682 on pages 25-26. We state that we did not have funding to analyze biomarkers, and this is a limitation of the study. In the following paragraphs we discuss the additional biomarkers that could be collected and analyzed in future studies.	See paragraph in discussion section beginning with, “Children with autism and ADHD are routinely diagnosed.....”
Reviewer 1: “What is the choice of parents?”	In the first paragraph of the discussion section beginning with line 553 on page 21 and ending on top of page 22, we discuss how parental dietary choices determine food preferences in children. We provide six new references on this aspect.	See paragraph in discussion section beginning with, “Parents play a crucial role in shaping a child’s food preferences because they choose what foods to feed their children [63].”
Reviewer 1: “In which way did the post-intervention results affect the diets of the children?”	We address this comment when we state in the paragraph beginning on line 602 on page 23, that one limitation of the study is that we did not collect diet data for the children.	See paragraph in discussion section beginning with “One limitation of this study is that we did not collect pre- and post-intervention food frequency data for the learning-disabled children.”
Reviewer 1: “Were there some measurable outcomes?”	Yes, the study outcomes are discussed in the Results section beginning on line 458 on page 18 and ending on line 551 on page 21. See Tables 6 and 7 for measurable outcomes, P-values showing significant changes in parental diet.	See conclusion paragraph in results section beginning with “The study results showed the use of an online nutritional epigenetics tutorial could facilitate and influence healthy dietary changes in parents of children with autism and ADHD.”
Reviewer 1: “Discussion section should be more robust.”	We’ve significantly expanded the discussion section. It is now robust. See Discussion section beginning on line 552 page 21. Begins with existing literature on what is known about parent diet and impact of ultra-processed foods on child development.	Discussion section begins with sentence “ Parents play a crucial role in shaping a child’s food preferences because they choose what foods to feed their children [63].

<p>Reviewer 2: “The paper needs distinct sections for the introduction, methods, results, and discussion.”</p>	<p>We follow the guidelines given by BPG for writing the results of a Basic Study. Guidelines for Authors: Basic Study https://www.wjgnet.com/bpg/GerInfo/218 Format for Manuscript Submission: Basic Study https://bsdwebstorage.blob.core.windows.net/bpggerinfo/Format_for_Manuscript_Submission-Basic_Study.pdf Guidelines for Manuscript Preparation and Submission: Basic Study https://bsdwebstorage.blob.core.windows.net/bpggerinfo/Guidelines_for_Manuscript_Preparation_and_Submission-Basic_Study.pdf</p>	<p>The guidelines require a background section, methods section, results section, and conclusion. Our BACKGROUND section begins on page 4, line 99. Our METHODS section begins on page 9 on line 235. Our RESULTS section begins on page 18. Our DISCUSSION section begins on page 21. Our short CONCLUSION paragraph is on page 28.</p>
<p>Reviewer 2: “Present the title so that it states the finding....Suggest “Nutritional Epigenetics Education Improves Parent Diet and Attitude.....”</p>	<p>According to the BPG guidelines the title can only be 18 words long. We have re-written the title so that it now includes the most important finding. The title is now “Nutritional epigenetics education improves diet and attitude of parents of children with autism or attention deficit/hyperactivity disorder.”</p>	<p>See new title on title page of manuscript- page 1.</p>
<p>Reviewer 2: “The abstract should mention the study design, primary outcome measures, key findings.” The abstract should not exceed 250 words.</p>	<p>According to the BPG guidelines, the abstract must be organized with the following sections: BACKGROUND (No more than 100 words) We meet this requirement. AIM: (No more than 20 words with purpose of study stated clearly) We meet this requirement METHODS (no LESS than 80 words, include basic study design, setting, number of participants, intervention, statistical methods used) We meet this requirement. RESULTS (no LESS than 120 words with key P-values for all significant findings) CONCLUSION (no more than 30 words) We meet this requirement stating findings in present tense.</p>	<p>See revised abstract with reviewer suggestions incorporated to the BPG requirements. Basic study design now given in methods section and Conclusion section includes main result in present tense with sentence starting “Here we show.....”</p>

<p>Reviewer 2: "List as many key words allowed by the journal from MeSH and use as many as possible in the title and abstract</p>	<p>We changed the key words. Only six key words are allowed by the journal. The keywords we selected were listed in MeSH.</p>	<p>We used the following six keywords with highlighted terms representing new key words:</p> <p>Epigenomics; Parenteral nutrition; Autism; Attention deficit/hyperactivity disorder; Ultra-processed foods; Heavy metals</p>
<p>Reviewer 2: The background section should be more structured so that the study's objective (aim) and hypothesis is made clearer. Include a discussion of the neural substrates or mechanisms creating conditions for autism and ADHD."</p>	<p>We rewrote the BACKGROUND so that the mechanisms underlying the relationship between ultra-processed food intake, heavy metal exposures, and the development of autism and ADHD are more clearly presented. We discuss the neural substrates that are impacted by exposures to lead (Pb) and mercury (Hg). We discuss the role of diet (e.g. nutrients, heavy metal exposures) in modulating gene activity and important substrates (metallothionein and paraoxonase). We make it clear that the consumption of ultra-processed foods leads to alterations in gene function creating conditions for the development of autism and ADHD. We clarified the aim of the study.</p> <p style="text-align: center;">AIM of study: The aim of this basic study was to test the efficacy of a six-week nutritional epigenetics tutorial in reducing parental ultra-processed food intake.</p>	<p>The new BACKGROUND section begins on page 4, line 99 and ends on page 9, line 228. Following the BACKGROUND section is the AIM statement as required by the BPG BASIC STUDY manuscript directions.</p>
<p>Reviewer 2: "Methods section needs short introductory paragraph."</p>	<p>We added a short introductory paragraph to the methods section beginning on line 235 on bottom of pg. 9 and ending on line 249 of pg.10</p>	<p>See new introductory paragraph to METHODS section.</p>
<p>Reviewer 2: The section on curriculum development lacks</p>	<p>Methods section: We created Table 1 that provides detailed information on the content covered in each module of instruction.</p>	<p>See Table 1.</p>

detailed information on content covered in each module.”		
Reviewer 2: Need more explanation on assigning participants to test and control groups.	Methods section: We added a sentence providing a description of how participants were alternately assigned to each group. See line 351 on page 14.	“Participants were alternately assigned to the test or control group when eligibility was confirmed and after receipt of the signed Informed Consent form.”
Reviewer 2: Size of sample	Methods section: We added a sub-section on how we determined the sample size. See line 301 and read to 326. New sub-section titled, “ Sample size calculation.”	Details of data used to determine sample size collection in Table 2. Read the sample size calculation section and see Table 2.
Reviewer 2: ..”more information on survey development , pilot testing or validation efforts.”	Methods section: We added a paragraph and a reference on the pilot testing and validation of the survey. See line 403 on page 16. Ref. [50].	See paragraph on page 16 beginning with “ The food frequency questions had been pilot tested successfully and validated in a previous clinical trial.
Reviewer 2: “specify type of questions used to measure dietary habits.”	Methods section: We provide details on the food frequency questions used in the survey along with a reference. Ref. [59]. See details in paragraph on line 371, page 14. Read through line 385 on page 15. All food frequency questions are provided on Tables 4 and 5.	Details on food frequency question wording begin with the sentence on page 14 “The food frequency questions were modeled after those used by the National Cancer Institute [59].....
Reviewer 2: “The data analysis section lacks detail on statistical methods and tests employed...”	Data analysis: Beginning on line 433 on page 17 we provide details on the statistical tests, including the assumptions made and the significance level chosen.	Please read that new section on page 17 that begins with the sentence “ Comparisons of pre- and post-intervention diet scores for test and control groups were performed using a two-sample paired t-test, with two tails and alpha = .05.

<p>Reviewer 2: “Presentation of results needs improvement with key findings given in a separate table.”</p>	<p>Results section: Tables 3-9 provide the results or observations made during the study. Tables 6 and 7 provide key findings with t-test results. Table 7 is a summary of the key findings with details on the tests we did.</p>	<p>Table 7 provides a summary of the results.</p>
<p>Reviewer 2: “Need paragraph at end of results section, with more general context.”</p>	<p>Results section: We added a paragraph at the end of the results section beginning on line 541 on pg. 21</p>	<p>New paragraph at end of results sections begins with “The study results showed the use of an online nutritional epigenetics tutorial could facilitate and influence healthy dietary changes in parents of children with autism and ADHD.....</p>
<p>Reviewer 2: Expand discussion; existing literature, implications of findings, limitations of study, cite more references</p>	<p>Discussion section: We’ve significantly expanded the discussion section. It is now robust. See Discussion section beginning on line 552 page 21. Begins with existing literature on what is known about parent diet and impact of ultra-processed foods on child development. Implications of the study’s findings discussed with new references provided. Suggestions for future research are given. Limitations of study are discussed fully to include bias in participant recruitment, lack of biomarker data, sample size, no data collected for child diet. 92 references.</p>	<p>Discussion section begins with sentence “ Parents play a crucial role in shaping a child’s food preferences because they choose what foods to feed their children [63].</p>

RESPONSE TO RE-REVIEW REPORT MANUSCRIPT NO. 88545

REVIEWER Comments	Our response	Location of changes in manuscript
<p>Reviewer Code: 05688164 Hungary - "I believe the manuscript meets the journal's high standards for publication. I am looking forward to seeing more papers written by the same authors."</p>	<p>Thank you so much for your review and positive feedback. We really appreciated the comments you provided and did our best to address your concerns. Thank you for accepting our revision and recommending publication.</p>	<p>NO changes requested by this reviewer.</p>
<p>Reviewer Code: 00573592 Italy - "If there are not outcomes pre and post intervention, how is it possible to ensure the reliability of results?"</p>	<p>Thank you so much for your comments. The manuscript is stronger now that we have addressed your concerns. Please see line 754 on page 28, in which we write the paragraph that gives details on the pre and post intervention outcomes. See Table 6 and Table 7 (located after the reference and figure sections near the end of the manuscript). We show the outcomes in Table 6. Table 7 shows the statistical analyses breakdown which shows the reliability of the results.</p>	<p>Paragraph on page 28 begins with " The pre- and post-intervention outcomes presented in Table 6 show the nutrition education program used in this study was an effective tool because parents who received the nutritional epigenetics instruction significantly decreased their ultra-processed food intake (P < .001) and significantly increased their whole and/organic food intake (P < .05). Table 7 provides details on the reliability of our outcome measurements from a statistical perspective.</p>

<p>Reviewer Code: 00573592 Italy - “Why is this nutritional model useful for parents of ASD and ADHD children and not for other pathologies or for non-affected people?”</p>	<p>Thank you for your comment. You raise a good point. This model can be used to address other pathologies associated with heavy metal exposures. We’ve added a couple more paragraphs to address your comment. Please read highlighted section beginning on line 725, page 27. Read through the next two paragraphs.</p>	<p>Paragraph on page 27 begins with The risk of heavy metal exposure from eating ultra-processed foods has been clearly demonstrated in studies conducted by Kahn et al. [88], Wells et al. [89], and Raehsler et al. [90]. Kahn et al. found heavy metal concentrations in food products significantly correlated with the heavy metal levels detected in human blood samples [88]. Wells et al. verified mercury exposure from non-fish food occurs through the consumption of vegetable oil, an ingredient commonly found in ultra-processed foodstuffs [89]. Raehsler et al. determined excessive intake of ultra-processed “gluten-free” food may lead to significantly higher levels of cadmium, lead, and mercury in the blood</p>
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[90]. The heavy metal exposures from drinking contaminated water, or eating ultra-processed food, will destroy the metabolic processes in the human body via oxidative stress [91]. The nutritional epigenetics model for autism and ADHD shows how this oxidative stress occurs (Figure 1) and may thus be a useful tool for understanding other pathologies associated with heavy metal exposures.

In addition to autism and ADHD, heavy metal exposures, especially cadmium, lead, and mercury, are positively associated with the development of atherosclerotic cardiovascular disease [92] and non-alcoholic fatty liver disease [93]. From a

		<p>toxicological perspective, it is interesting to note that non-alcoholic fatty liver disease is associated with ultra-processed food intake in a dose-response manner similar to the dose-response relationship showing heavy metal toxicity [94]. Not surprisingly, numerous pathologies are associated with ultra-processed food intake [95]. In a recent review, Elizabeth et al examined forty-three articles to determine any associations between ultra-processed food intake and adverse health outcomes [95]. Of the forty-three articles, thirty-seven found excessive ultra-processed food intake was associated with at least one of the following pathologies: obesity, overweight, cancer, type-2 diabetes,</p>
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		<p>depression, irritable bowel syndrome, cardiovascular disease, and all-cause mortality [95]. Any nutrition education program that helps individuals significantly reduce their intake of ultra-processed foods will be useful because evidence suggests that switching to a healthy diet will prevent disease and/or improve health outcomes [96].</p>
<p>Reviewer Code: 00573592 Italy “ What is the link between ASD and the model if the effectiveness of the model has been applied only on parents?</p>	<p>Thank you for your comment. More research needs to be done. We are providing effective intervention tools for future studies. Please read beginning on line 759 – this is how we address your comment.</p>	<p>Begin reading “Parent nutritional epigenetics instruction is a novel nutrition education intervention because evidence suggests that if parents reduce their consumption of ultra-processed foods, then their children will also reduce their consumption of ultra-processed foods [67, 68]. Using the nutritional epigenetics model as a</p>

		<p>teaching tool for helping parents reduce their consumption of ultra-processed foods may lead to healthier home food environments and subsequent improvements in child diet by reducing the heavy metal exposures associated with autism and ADHD. More research is needed to verify the reductions in heavy metal exposures that may be associated with reducing ultra-processed food intake.</p> <p>Meanwhile heavy metal residues continue to be a problem in the American food supply. The US Congress recently released two reports on the problem of heavy metals, including inorganic mercury (I-Hg) and PB, in baby foods sold in America [97, 98]. Dietary heavy</p>
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		<p>metal exposures, I-Hg and Pb, are an important construct in the nutritional epigenetic model for autism and ADHD (Figure 1). In addition to collecting and analyzing blood samples for Hg and Pb, changes in MT and PON1 gene activity levels could also be measured in children with autism and ADHD pre- and post-parental nutritional epigenetics instruction. Meguid et al. successfully measured changes in the genetic expression of MT-1 in children with autism after zinc supplementation [99]. Numerous studies have already been conducted successfully to measure PON1 gene activity in response to changes in diet [100]. Further studies that use nutritional epigenetics</p>
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		<p>instruction to modulate diet could shed light on the role ultra-processed foods [and heavy metal exposures] play in the development of autism and ADHD via MT gene disruption or PON1 gene suppression [101].</p>
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