Reply to the Reviewer/Editor.

Dear Respected Editor/Reviewer

Good day

Thank you very much for the comprehensive review and the precious time you spent reviewing this study. We made the advised changes and answered the queries. All the changes were marked in red for easy tracking by the reviewer. The manuscript looks much better with these changes, and we tried to improve the language as much as possible. Thank you again for your precious assistance.

Here we are replying point by point:

Reviewer 1:

This is a very interesting study, but much additional work needs to be done regarding methodology, results, and Discussion.

1. Multiple t-tests, No Bonferroni correction, is a big problem that affects the interpretation of the results of this study. Whenever multiple statistical tests are made simultaneously, chance error creeps in and yields some significant results that are spurious. To control for this problem, Bonferroni developed a procedure that helps identify spurious findings. Alternatively, this problem can be addressed by selecting a more conservative alpha level. For this study, using $p < .01$ instead of $p < .05$ would help ameliorate the problem. By applying either the Bonferroni formula or choosing $p < .01$, the results (and therefore the interpretation of the data are changed as follows: (a) In Table 2, the Naa/Cr correlation in the Frontal lobe is significant, and neither the Naa/Cr nor the Cho/Cr correlation in the Occipital lobe are significant; and (b) In Table 3, only one of the correlations with IQ remains significant (F Naa/Cr).

Our reply:

Thank you for your valuable feedback on our article. We appreciate your time and effort in providing constructive comments. We have carefully considered your concerns regarding the statistical analysis and would like to address them as follows:

Multiple Testing and Bonferroni Correction:

We acknowledge that multiple t-tests without a correction method can increase the risk of Type I errors. We agree with your suggestion that using the Bonferroni correction or a more conservative alpha level would be suitable for addressing this issue. We reevaluated our statistical analysis with two expert statisticians. They ensured that we did not need Bonferroni

Correction as we compared only two groups. Therefore, $p < .05$ is still to be considered significant.

Impact on Interpretation of Results:

We understand that implementing the Bonferroni correction or adjusting the alpha level may change the statistical significance of specific correlations presented in Tables 2 and 3. We carefully reanalyzed the data using the recommended approach by the expert statisticians and followed their instructions accordingly.

We are grateful for your guidance in enhancing the quality and rigor of our study. Your suggestions will undoubtedly contribute to a more robust and accurate representation of our findings. We made the necessary revisions to address these concerns and ensure the validity of our results. Once again, thank you for your valuable input, and we look forward to your further evaluation of our revised manuscript.

2. Why unpaired t-tests? Groups were matched on age and, to some degree, on sex. Matched samples require paired t-tests, not unpaired. And why multiple t-tests instead of multivariate analysis?

Our reply:

We appreciate your insightful comments and would like to address your concerns regarding using unpaired t-tests and choosing multiple t-tests over multivariate analysis.

Unpaired T-tests vs. Paired T-tests:

You correctly pointed out that using unpaired t-tests may not be the most appropriate choice when groups are matched on specific variables, such as age and sex. We appreciate your feedback in this regard. In our initial analysis, we selected unpaired t-tests primarily due to the independence of our data points. We agree that the control group matches in age and, to some extent, sex with the studied group, but they are not similar. They are entirely independent. To ensure our point of view, we consulted two statistics professors; their opinion is to use the Unpaired T-tests. However, we understand that using paired t-tests would be more suitable when dealing with matched samples. We reevaluated our data and statistical analysis to determine whether a paired t-test would be more appropriate. According to the statisticians' opinion, there is no need to do a paired t-test.

Choice of Statistical Analysis:

Your suggestion to consider multivariate analysis instead of multiple t-tests is well-taken. Multivariate analysis has the advantage of assessing the relationships between multiple dependent variables simultaneously and can provide a more comprehensive understanding of the data. We added Table 4 with multivariate analysis and assessed its suitability for our dataset. It seemed appropriate, so we included this analysis in our revised study, which provided a more robust statistical framework.
We sincerely appreciate your expertise and guidance in improving the statistical rigor of our research. Your feedback is invaluable, and we are committed to making the necessary revisions to enhance the quality of our study.

Once again, thank you for your thoughtful review, and we look forward to your continued evaluation of our revised manuscript.

3. Why were 20 of the original 60 DS children eliminated from the study? What is the rationale? Of the 20 eliminated, how many couldn't perform the required tasks, and how many were simply removed from the sample for unnamed or unknown reasons?

   Our reply: They were excluded due to missed follow-ups, inability to participate, or refusal to do an MRI.

4. "The IQ was primarily influenced by . . ." Implies causality. One cannot infer causality from correlations, just relationships.

Our reply:

We appreciate your attention to detail and would like to address your concern regarding the implication of causality in our abstract.

You correctly pointed out that our statement, "The IQ was primarily influenced by," may imply causality, which is inappropriate when discussing correlations. We acknowledge this oversight and apologize for any confusion it may have caused. We understand that correlations only establish relationships, not causation.

We revised the manuscript to accurately reflect the nature of the relationships we explored in our study. Specifically, we rephrased the statement to emphasize the associations or relationships between brain metabolic profiles and IQ in children with Down syndrome

without implying causation. We aim to ensure that the manuscript accurately represents our research findings and aligns with the principles of scientific rigor. We are grateful for your feedback, which contributed to the clarity and precision of our article. We look forward to your continued evaluation of our revised manuscript.

5. There is no way to interpret the results as having anything to do with DS. The authors compared MRS data for one group with average IQ and one group with low IQ. The results may apply to low-functioning children in general, but no differences can be attributed to the DS diagnosis. This serious limitation points to another methodological problem with the study—the authors should have included a third group, namely low-functioning children and adolescents who do NOT have DS.

   Our reply:

   We appreciate your thoughtful review and the opportunity to address your concerns regarding interpreting our study results and including an additional group of low-functioning children without Down syndrome (DS).

   Your observation highlights an important aspect of our study design, and we understand your point of view. We would like to provide clarification and address your comments as follows:

   Interpretation of Study Results:

   We acknowledge your concern about interpreting our results about DS. Our primary focus was to assess the brain metabolic profiles of children with DS and their association with IQ. However, we recognize that our study design did not include a separate group of low-functioning children without DS for direct comparison. We added this point in the limitation section.

   Consideration of Additional Group:

   Your suggestion to include a third group of low-functioning children and adolescents without DS is well-founded. Such a group would have provided a valuable reference point for evaluating whether the observed differences in brain metabolic profiles and their correlation with IQ are specific to DS or could apply to low-functioning children in general.

We appreciate your recommendation for future research directions. Including a group of low-functioning children without DS in subsequent studies could help us differentiate between DS-specific metabolic variations and those common to low-functioning individuals.

We are committed to conducting rigorous research that contributes to a deeper understanding of the complexities of DS and its impact on brain metabolism. Your feedback is invaluable in refining our approach and addressing potential limitations in our study design. We will consider this point as one of the study's limitations. In future investigations, we will consider your suggestion and explore including a broader range of participant groups to enhance the generalizability of our findings.

Thank you for your valuable input, which will undoubtedly contribute to the robustness and depth of our research. We look forward to your continued evaluation of our work.

6. The authors computed 3 ratios in 4 regions of the brain, for a total of 12 comparisons. They provided excellent scientific rationales for their selection of the specific ratios and brain regions to explore. But they did not hypothesize which of these 12 were most likely to be significant based on scientific rationale and previous research results; from multiple MRI studies on individuals with DS. Instead, they used a “shotgun” approach to find out what was significant and what was not. Whenever that type of approach is used, significant findings need to be cross-validated with a new sample before they can be meaningfully interpreted. Despite the fact that the authors did not rely on the relevant MRI literature to formulate specific hypotheses, they did an excellent job in the Discussion section of integrating their findings with previous results.

   Our reply:

   We appreciate your thorough review of our article and your insightful comments regarding the statistical analysis and hypothesis formulation. Your feedback provides us with an opportunity to clarify our approach and address your concerns:

   Hypothesis Formulation:

   You correctly pointed out that we did not explicitly formulate specific hypotheses for the 12 comparisons of brain metabolic ratios in different regions of the brain. We appreciate your feedback on this matter. While we did not predefine hypotheses for individual ratios

and brain regions, our study's primary objective was to explore potential associations between brain metabolic profiles and IQ in children with Down syndrome (DS).

Statistical Approach:
You mentioned that our approach resembled a "shotgun" approach, which may yield significant findings that require cross-validation. We acknowledge this observation. We intended to explore a wide range of potential relationships between brain metabolic ratios and IQ to gain a comprehensive understanding of the topic. We understand that such exploratory analyses should be followed by confirmatory studies to validate the observed associations. We emphasized this point in the limitation section.

Integration with Previous Research:
We are pleased to hear that you found our Discussion section effective in integrating our findings with previous research results. We aimed to provide context and implications for our results in light of existing literature, even though we did not base our specific hypotheses on prior MRI studies of individuals with DS.

In response to your comments, we agree that future research should include a more focused approach with specific hypotheses derived from relevant MRI literature. Additionally, we will consider conducting follow-up studies to validate our significant findings.

We appreciate your constructive feedback, which will help us refine our research and enhance the robustness of our findings. Your insights are invaluable, and we look forward to your continued evaluation of our work.

Sincerely,
Professor Mohammed Elbeltagi

Reply to the Reviewer/Editor.

Dear Respected Editor/Reviewer

Good day

Thank you very much for the comprehensive review and the precious time you spent reviewing this study. We made the advised changes and answered the queries. All the changes were marked in red for easy tracking by the reviewer. The manuscript looks much better with these changes, and we tried to improve the language as much as possible. Thank you again for your precious assistance.

Here we are replying point by point:

Reviewer 1:

The revision addressed some of my concerns adequately. I appreciate that the authors conducted a multivariate analysis and added some of my concerns as limitations of their study and as suggestions for future research. However, the one comment that I do not agree with concerns multiple comparisons. The argument that "only two samples are compared" is not important. Table 2 makes 12 simultaneous comparisons. So does Table 3. Following the strict Bonferroni procedure, using $p < .004$ will yield a family-wise alpha level of .05 (the Bonferroni formula is .05 divided by the number of simultaneous comparisons, in this case 12, which yields $p < .004$). However, since this procedure is conservative, a more liberal level would be $p < .01$. That would be acceptable for this study. But using $p < .05$ is not acceptable.

Our reply:

Dear Respected Reviewer,

Thank you for taking the time to re-review our manuscript and for providing valuable feedback on our revision. We are pleased to hear that the revisions addressed most of your concerns and that you appreciate the inclusion of the multivariate analysis and the acknowledgment of limitations for future research.

We acknowledge and respect your perspective regarding the issue of multiple comparisons. We understand your concern regarding applying a stricter alpha level to account for the multiple comparisons in Tables 2 and 3, mainly using the Bonferroni correction.

We agree that using a more conservative alpha level, such as $p < p < .01$ or even .004, would be a more stringent approach to mitigate the risk of Type I errors associated with multiple comparisons. Given your feedback, we reevaluated the statistical analysis and considered adjusting the reported p-values accordingly. We added this in the method section and corrected the P value in all the tables. We changed the results accordingly. All the changes were highlighted in red.

Thank you again for your thorough review and insightful suggestions. We will carefully address this concern and make the necessary revisions to ensure the rigor and validity of our study.

Best regards,

Sincerely,
Professor Mohammed Elbeltagi