

June 20, 2014



Dear Editor,

Enclosed please find the edited manuscript in Word format (file name: WJG 2014-11492, revised.doc).

**Title:** Increased circulating zonulin in children with biopsy-proven nonalcoholic fatty liver disease

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**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 11492

The manuscript has been revised according to the suggestions of reviewers:

Reviewer # 00038192

- Abbreviations in the abstract have been explained;
- We have corrected the spelling and grammatical errors on pages 5 and 11, as well as throughout the manuscript;
- We apologize for the mistaken units of fasting glucose on table 1; now they read “mg/dL”;
- Since in the present study no significant association has been found between zonulin and inflammation as well as between zonulin and fibrosis, we prefer not to encumber the revised manuscript with further graphs.

Reviewer # 00069701

1. We have included the full name of BMI-SD score when it first appears in the manuscript;
2. There are no studies on circulating zonulin in patients with NAFLD. The previous study by Miele *et al.* demonstrated an increased intestinal permeability and tight junction (TJ) alterations in 35 adult patients with biopsy-proven NAFLD compared with healthy adult subjects. Zonulin was the first TJ-associated protein to be identified, and it is now widely considered to be an excellent marker for detecting intact cell-to-cell contacts and assessing TJ integrity. Serum or plasma zonulin concentrations are considered useful markers of intestinal permeability. This is the reason why we chose zonulin. In contrast, as discussed in the “Discussion Section” reliability of the lactulose-mannitol bowel permeability test may be affected by many clinical factors including BMI;
3. We have appended the full name of MET to the footnote of table 2;
4. As suggested by the reviewer, we have incorporated the definition of mild, moderate, and severe steatosis according to the NASH Clinical Research Network (CNR), and have included the number of patients belonging to each group (legend to figure 2);
5. We have discussed potential mechanisms underlying the association between zonulin as mediator of intestinal permeability and NAFLD(P.10-12);
6. We have corrected the spelling and grammatical errors throughout the manuscript.

Reviewer # 00053423

- As suggested by the reviewer, we have included in a third column the P values for

comparison between children with and without NAFLD (rather than appending them to the footnote of table 1); and have included the results of a multivariate analysis performed to determine the independent impact of NAFLD on circulating zonulin after adjustment for potential confounding variables including adipose tissue parameters and glucose metabolism parameters;

- The reviewer is surprised by the negative findings of the relation between zonulin concentrations and BMI-SDS, VAT, and SAT. In univariate analysis (data not shown), we found a significant relationship between zonulin concentrations and BMI-SDS, VAT, and SAT, but they were no longer significant after adjustment for age, gender, and pubertal status. In adult obese patients with and without glucose intolerance, Moreno-Navarrete *et al.* (ref. # 16) also found that circulating zonulin was strongly associated with BMI only in univariate analysis. Contrary to Moreno-Navarrete *et al.*, ZaK-Golab *et al.* assessed plasma zonulin in relation to composition of gut microbiota in obese and normal weight subjects (ref. # 15). They showed a positive correlation between BMI and zonulin. Therefore, differences in patients' clinical and metabolic characteristics may explain differences among the studies;
- We have discussed in more detail possible reasons accounting for the different results between our study and those of Miele *et al.* and Giorgio *et al.*;
- As suggested by the reviewer, we have clarified in the revised manuscript that the cross sectional design of the study precludes the establishment of a cause-effect relation between increased zonulin concentrations and severity of steatosis and the presence of NASH.

Thank you again for considering our manuscript for the *World Journal of Gastroenterology*.

Sincerely yours,  
Claudio Chiesa, MD  
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