Dear Dr. Y-J Ma, the Director of Editorial Office of WJG,

Subject: Submission of revised paper Ms. No. 36189; Title: „Intestinal parameters of oxidative imbalance in celiac adults with extraintestinal manifestations"

Thank you for your email dated 19 October 2017 enclosing the reviewers' comments. We appreciate the decision of Editorial Office of WJG, giving us the chance to revise this manuscript after consideration of Reviewers critical comments. We have carefully reviewed all Reviewers critical comments and suggestions and have revised the manuscript accordingly. Now we would like to submit the revised version of Ms. No. 36189R1 along with a point-by-point response to all comments raised by Reviewers (please see below). The changes to the manuscript in revised version are tracked for proper identification how we have dealt with Reviewers comments.

We hope that we did our best to revise this manuscript in accordance to Reviewer comments and we will look forward to hear for the decision on suitability of this work on important clinical problem for publication in your esteemed Journal.

Best regards,

Sincerely,

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Response to Reviewers Comments

We would like to thank the Reviewers for their deep and thorough review. We greatly appreciate Reviewer's constructive comments that helped us to revise the manuscript according to their suggestions and advises. Hereby, we provide our point-by-point reply to critical comments along with the identification of the changes made to the text of this paper in its original version.

Point-by-point response to Reviewer # 1 Comments

Comment: paper well prepared my only issue is the title that not follow the rest of paper

AU: All Authors are grateful to this Reviewer for her/his positive and encouraging comments. As suggested by this Reviewer, the title of the manuscript has been changed for better addressing of the nature of this study into "Intestinal parameters of the oxidative imbalance in celiac adults with extraintestinal manifestations".

Point-by-point response to Reviewer # 2 Comments

Comment 1: In this study the Authors present their results of the magnitude of the effect of oxidative stress in adult patients with celiac disease. The manuscript is complete and well written. The topic is of major interest to a large readership, although it is not novel. However, the paper has the following flaws:

1. The title is not reflected in the abstract nor in the core tip; that's, the studied subjects are celiac patients with extraintestinal manifestations. This should be specified both in the "aim" and the "methods" sections of the abstract, as well as in the core tip.

AU: As per your suggestion, we have revised the title of this work and mounted now the specific information on studied celiac patients with extraintestinal manifestations in both, the text of "aims" and the "methods" sections of the abstract as well as the core tip.

Comment 2: The sample size may be too small to draw valid conclusions. A type 2 statistical error could undermine the results.

AU: We agree that the relatively small number of patients in each celiac subgroups could be a drawback of this study. We made this issue clear in the study limitations paragraph before the last section of final study conclusions by the end of Discussion. Actually, the number of patients reflected a real number of celiac patients admitted for data collection and followed up throughout last three years in our unit. The study is not closed up and will be continued, since we are planning further research with higher number of enrolled subjects to support our present observations.

Comment 3: The introduction section is too long. A couple of paragraphs should be deleted.

AU: As you suggested, the Introduction Section has been shortened by skipping the paragraphs less relevant to the major topic of this study. The references had been changed into a new order accordingly, because of responding to queries raised by another Reviewer.

Comment 4: The clinical characteristics of study subjects (with emphasis on their specific extraintestinal manifestations) should be added to the demographic data (table 1) or described in a separate table.

AU: Again, we are grateful for this suggestion. The clinical characteristics of study subjects have been described in a separate table - Table 2.

Comment 5: The Authors state that the study is prospective; actually this is a case-control study, and not a prospective study.

AU: We have considered your remarks on the type of our MS (prospective *vs.* case-controlled) and accepted your conclusion that case-control study better addresses the type of our present work. This information has been included in the manuscript.

Comment 6: The discussion section should start by stating the main results and their significance, rather than with a repetition of what was already stated in the introduction section. Also, the Authors state" We explored, for the first time, the role of serum uric acid as a non-enzymatic plasma antioxidant in adult patients with CD"; this statement is inaccurate, as the topic has been previously tackled and published by several authors.

AU: You are again right stating that the Discussion should present the major finding starting from its beginning, and therefore we have modified it. Regarding your

comment on uric acid, we also agree with you that this metabolite has been tackled in some studies in the past. However, experimental and clinical studies have confirmed antioxidant activity of uric acid *in vivo* (these publications have been used in discussion), while the clinical evidence on uric acid as the potential biomarker of the oxidative stress, especially in adult celiac patients, are sparse. Nevertheless, we have tempered our previous statement starting from words "...We explored, for the first time....." stating now more objectively that: ".... There is increasing experimental and clinical evidence on uric acid acting as important antioxidant... [42]....."

Comment 7: The study limitations (e.g. sample size, self-reported adherence to GFD,...) should be stated clearly before the conclusions.

AU: We appreciate your important comment. As suggested, the study limitations have been stated before the conclusion. In this paragraph we refer to small size of samples, self-reported adherence to GDF and the persistence of morphologic impairment of duodenal mucosa of celiac patients despite much faster fall in antibodies titer observed in GFD patients.

Comment 8: The conclusions are somehow weak. The Authors should expand on the potential that several nutrients exert antioxidant effects and influence gene expression, therefore reprising a useful approach for nutritional intervention in CD subjects. Additionally, the Authors should discuss the usefulness of nutritional genomics as a tool for targeted medical nutrition therapy, and possibly suggest further basic *research, extensive epidemiological studies and controlled intervention trials.*

AU: Again your suggestion was excellent and we have made some attempts to modify the study conclusions accordingly. We have now discussed the potential of certain nutrients with antioxidant activity as a useful approach for the nutritional intervention in CD subjects. Furthermore, we have emphasized the usefulness of nutritional genomics as the tool for targeted medical therapy and development of individualized medicine, all these aspects that require a further research.

Point-by-point response to Reviewer # 3 Comments

(1) Is the overall structure of the manuscript complete? A complete manuscript will contain title, abstract, key words, introduction, materials, methods, experimental procedure, results, discussion, conclusion, acknowledgements, and references. YES

AU: We are grateful for your expertise.

(2) What is the scientific question proposed in the manuscript? This should be clearly presented in the Introduction section, along with the pertinent background, rationale, aim, major findings and potential significance of the study. Collectively, this information should inform whether the manuscript would be interesting enough to warrant readers' attention? THE AIM OF OUR STUDY WAS TO DETERMINE THE INVOLVEMENT OF OXIDATIVE STRESS IN THE MECHANISM OF MUCOSAL INJURY OF THE SMALL INTESTINE AND TO ASSESS THE EFFECT OF OXIDATIVE STRESS ON THE COURSE OF CD IN ADULT PATIENTS WITH NON-CLASSIC SYMPTOMS AND EXTRAINTESTINAL MANIFESTATIONS.

AU: This is exactly what our paper is talking about.

(3) Which special (unique, innovative and/or timely, appropriate) methods and techniques are adopted in the manuscript? This should be clearly presented in the Methods section. In addition, does the manuscript provide adequate details of methods (including experimental design, subjects or materials, data collection methods, and statistical methods) to allow a reader to repeat the research? THE EXPRESSION OF IL-1B, TNF-A, IL-10, HSP-70, HIF-1A, SOD, AND BAX TRANSCRIPTS IN HUMAN SAMPLES WAS DETERMINED BY RT-PCR.

AU: Reviewer picked up the special message we wanted to address since these issues have not been extensively explored in adult celiac patients.

(4) Is the source of the data that is presented reliable? This will be indicated by the information presented in the Results section. The information in the results section will also indicate the academic significance of the main findings (including figure and tables). YES

(5) What are the results obtained from the data that is presented in the manuscript? This information will make up the Discussion section. It will also answer the questions of whether the results answered the proposed scientific question, achieved the aim of the study, or confirmed or rejected the hypothesis proposed in the manuscript. The results of biochemical tests are presented in Table 3. WHAT ABOUT t-TG / EmA STATUS OF CD PATIENTS IN GFD? WHY A LOT OF CD PATIENTS WERE NOT ON GFD? A TOO BIG PERCENTAGE OF CD PATIENTS ON GRD HAVE A GREAT DUODENAL DAMAGE (40% HAVE A MARSH 3 DAMAGE -> THEIR RESULTS CAN'T BE CONSIDERED WITH THE GROUP OF CD PATIENTS RESPONSIVE TO GFD.

AU: We are grateful to Reviewer for this comment and we agree with this expertise. It should be noted that in our study, the duodenal damage persisted in quite a large percentage of patients with CD on GFD despite evident decline in celiac antibodies and the overall clinical improvement. The main criteria for inclusion into this group involved a detailed assessment by an experienced gastroenterologist and dietician of a proper dietary adherence, clinical recovery and predominantly, the analysis of the negativity of serologic markers (TGA/EmA). Moreover, we were aware about previous observations in literature that histology do not always accompanies the decline in serologic markers. Our present observation extends these information's and seem to be consistent with the results of other studies [please see new references # 32, # 33 and # 35] on much more delayed healing and recovery of microscopic damage in celiac patients compared with the faster timing in a disappearance of antibodies. The results of serum levels of celiac antibodies (TGA/EmA) are presented in Table 5. Our study involved 22 patients with CD who did not completely adhere to GFD as indicated by the specializing team assessment, which was reflected in the positive serology. Although this treatment with GFD should result in a faster recovery from both clinical symptoms and intestinal damage, some patients suffer from serious impairment of their quality of life, due to their stigmatization and segregation by switching them to special diet. In fact, mostly in adulthood, the establishment of a strict GFD has sometimes personal difficulties to overcome, from both a practical and psychological point of view and finally, those patients were not enrolled to GFD group. To conclude, your comment was

meaningful, therefore, we have cited now abovementioned relevant references regarding this issue.

(6) What are the conclusions of the manuscript? These should be clearly presented in the Conclusion section. In addition, the section should present the contributions of the conclusions to the field and the weaknesses of the study, and provide future research directions. IN CONCLUSION, BY ITS ASSOCIATION WITH INTESTINAL DAMAGE, THE COURSE OF THE DISEASE, AND PERHAPS EXTRAINTESTINAL DISORDERS, OXIDATIVE IMBALANCE APPEARS TO BE ONE OF THE MAJOR FACTORS IMPLICATED IN THE PATHOGENESIS OF CD.

AU: In the conclusion section, we have also discussed the potential, useful role of certain nutrients with antioxidant activity in nutritional intervention on CD subjects. However, we stressed out that the pathomechanism of CD with respect to several aspects including i.e. oxidative stress, mucosal healing incompatibilities with the levels of serologic markers, requires further research.

(7) Does the manuscript cite all important, relevant and timely references? NO

AU: We have updated references, especially referring to HSP examples in literature, you kindly provided us with. Many thanks indeed.

(8) Is there any indication of academic misconduct in the manuscript? NO

(9) Does the manuscript conform to the academic rules and norms and include a human and animal rights statement, institutional review board statement, informed consent statement, clinical trial registration statement, institutional animal care and use committee statement, animal care and use statement, biostatistics statement, and conflict-of-interest statement? YES

(10) Does the manuscript describe any important new methods, problems in or directions of research? YES

(11) Does this manuscript contribute to understanding the pathogenesis of disease, disease diagnosis, and treatment or prevention? YES

(12) Does the title of the manuscript contain key words; and is the title interesting enough to attract readers' attention? YES

(13) Does the topic of the manuscript fall within the scope of World Journal of Gastroenterology? YES

(14) Does the language of the manuscript reach the standard of publishing? YES

Reviewers' conclusions

(1) What are the new visions that the manuscript offers to readers? A DEEPENING OF THE ROLE OF OXIDATION PRODUCTS ON THE CD.

AU: Reviewer conclusion based on our findings is directly to the point.

(2) Are there any weaknesses or deficiencies in the manuscript? WHAT ABOUT t-TG / EmA STATUS OF CD PATIENTS IN GFD? WHY A LOT OF CD PATIENTS WERE NOT ON GFD? A TOO BIG PERCENTAGE OF CD PATIENTS ON GRD HAVE A GREAT DUODENAL DAMAGE (40% HAVE A MARSH 3 DAMAGE -> THEIR RESULTS CAN'T BE CONSIDERED WITH THE GROUP OF CD PATIENTS RESPONSIVE TO GFD) a) "The immune disturbances play a critical role in the pathogenesis of CD[15], but there have been no studies on the role of heat-shock proteins (HSPs) in the development of this disorder." THIS SEEMS NOT TRUE

PROTEIN-PROTEIN INTERACTION NETWORK OF CELIAC DISEASE. GASTROENTEROL HEPATOL BED BENCH. 2016 FALL;9(4):268-277.

AUTOIMMUNITY TO HEAT SHOCK PROTEINS AND VITAMIN D STATUS IN PATIENTS WITH CELIAC DISEASE WITHOUT ASSOCIATED DERMATITIS HERPETIFORMIS. J STEROID BIOCHEM MOL BIOL. 2017 OCT;173:23-27.
DISTINCT AND SYNERGISTIC CONTRIBUTIONS OF EPITHELIAL STRESS AND ADAPTIVE IMMUNITY TO FUNCTIONS OF INTRAEPITHELIAL KILLER CELLS AND ACTIVE CELIAC DISEASE. GASTROENTEROLOGY. 2015 SEP;149(3):681-91.E10.

INCREASED HEAT SHOCK PROTEIN 72 EXPRESSION IN CELIAC DISEASE. J PEDIATR GASTROENTEROL NUTR. 2010 NOV;51(5):573-8.

HEAT-SHOCK PROTEIN 70-1 AND HLA CLASS II GENE POLYMORPHISMS ASSOCIATED WITH CELIAC DISEASE SUSCEPTIBILITY IN NAVARRA (SPAIN). HUM IMMUNOL. 2001 AUG;62(8):821-5.

EXPRESSION OF HSP-65 IN JEJUNAL EPITHELIAL CELLS IN PATIENTS CLINICALLY SUSPECTED OF COELIAC DISEASE. AUTOIMMUNITY. 1999 OCT;31(2):125-32.

AU: As mentioned above the microscopic alterations in the duodenum of some CD patients on GFD did not heal within the time frame of disappearance of antibody titers. We notified a drop in plasma antibodies that preceded the healing of duodenal damage and full recovery from these lesions observed microscopically. In response to your question why a lot of CD patients were not on GFD we wanted to explain that in our study 22 patients with CD failed to completely adhere to GFD as indicated by assessment performed by the team of gastroenterologist and dietitian and confirmed by a positive serology. One should expect that GFD would have provided a full resolution of clinical symptoms as manifested by mucosal healing and fall in serum antibodies. However, in our study, duodenal damage persisted in some percentage of GFD patients despite decline in celiac antibodies and clinical improvement observed in these group of patients. This indicates that some CD patients do not always follow study guidelines including diet requirements because in their understanding, the diet may seriously affect their quality of life causing their stigmatization and segregating them from others. In the he adulthood, this raises a apparent concern for the establishment and realization of a strict GFD by these patients, from both a practical and psychological point of view. To support the notion about incompatibility of the healing with changes in antibody titers, we now cite a new published evidence by Lauzini *et al* [32], Lebwahl *et al* [33] and Wahab *et al* [35] on slower and incomplete healing mucosal recovery observed in celiac patients. We have added more updated citations on HSP, you kindly suggested to us, for which we are really grateful. However, we are under impression that the studies on HSP as potential markers of oxidative stress, especially in adult celiac patients with extraintestinal manifestations, the one of the major topic of our present study, are

sparse. Furthermore, most of the studies so far have focused on CD children than adults with CD and we refer to that point in the text of this revised version.

(3) Can the experiences and lessons presented in the manuscript improve the readers' practice? LITTLE

(4) Does the content of the manuscript have value for publication? If not, rejection should be recommended YES, BUT AFTER THE DISTINCTION BETWEEN CD PATIENTS ON GFD WITH HISTOLOGICAL DAMAGE OF MARSH 3 FROM THOSE WITH HISTOLOGICAL MARSH 0 OR 1

AU: Thank you for a general positive opinion about our work despite some critical issues. As specified above in our point-by-point response letter to you, we have made distinction between the time of the antibodies disappearance and incomplete mucosal healing in the revised version of our paper. In addition, we cited now 3 new references underlying this incompatibility between slower of the intestinal lesions in adult CD patients and serologic markers. We would like to explain, that the main criteria for inclusion in this group involved a specialist assessment of proper dietary adherence, clinical recovery and above all, the negativity of serologic markers (TGA/EmA) but not a complete healing of duodenal lesions. These observations seem consistent with the results of other studies [32, 33, 35]. However, we have mentioned objectively that further research is needed for better insight into mechanism of these alterations observed in adult celiac patients.

(5) Is the manuscript concise, clear, comprehensive, and convincing? YES, APART FROM THE CRITICISM I DISCUSSED ABOVE

AU: We considered all your critical comments according to our best knowledge to revise the text and improve this MS in its original form by providing some explanations as you requested.

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