

RE: Revision of the manuscript NO: 77800, entitled " Prevalence of Functional Gastrointestinal Disorders in children with Celiac Disease on different types of gluten-free diet"

Dear Editors,

Thank you very much for reviewing our paper.

As you suggested, we are sending you a revised version of the manuscript entitled: "Prevalence of Functional Gastrointestinal Disorders in children with Celiac Disease on different types of gluten-free diet".

We have carefully read the comments written by reviewers and have attempted to clarify the various shortcomings to the best of our ability.

We have included a list of explanations for each comment, listed under the headlines "Response to Reviewer 1-2-3 Comments"

We hope that this will be sufficient, but please let us know if there are other problems that need to be addressed.

Once again thank you for your help and consideration.

Sincerely

Caterina Strisciuglio

## Response to Reviewer 1 Comments

We thank the reviewer for the constructive and positive comments and suggestions, and we herein provide a point-to-point response

The study was well designed, evaluated the control of different types of gluten free diet on celiac disease in children, and reached interesting conclusions. I have two questions. First, whether the author can provide the original experimental data. The original data is not provided in the attachment of the article.

**Response 1: We thank the reviewer for this careful observation and we we have attached a supplementary data the experimental data requested.**

| <b>N = 104</b>        |             |             |
|-----------------------|-------------|-------------|
| <b>DIET</b>           |             |             |
|                       | Natural     | 55 (53%)    |
|                       | Not natural | 49 (47%)    |
| <b>KCAL</b>           |             | 1,397 (350) |
| <b>PRO</b>            |             | 51 (14)     |
| <b>FAT</b>            |             | 61 (19)     |
| <b>CHO</b>            |             | 179 (106)   |
| <b>STARCH</b>         |             | 37 (33)     |
| <b>CHOL</b>           |             | 145 (86)    |
| <b>SATURATED F.A.</b> |             | 14.5 (6.5)  |
| <b>POLY. F.A.</b>     |             | 4.53 (1.97) |
| <b>MONO. F.A.</b>     |             | 23 (11)     |
| <b>CA</b>             |             | 416 (203)   |
| <b>NA</b>             |             | 834 (615)   |
| <b>K</b>              |             | 1,345 (572) |
| <b>P</b>              |             | 663 (243)   |
| <b>FE</b>             |             | 4.58 (2.51) |
| <b>ZIN</b>            |             | 5.21 (2.27) |
| <b>FOLIC A.</b>       |             | 102 (74)    |
| <b>NIACIN</b>         |             | 7.9 (4.3)   |
| <b>RIBOFLAVIN</b>     |             | 0.86 (0.50) |
| <b>THIAMINE</b>       |             | 0.51 (0.22) |
| <b>VIT.A</b>          |             | 356 (208)   |
| <b>VIT.B6</b>         |             | 0.84 (0.50) |
| <b>VIT.C</b>          |             | 64 (72)     |
| <b>VIT.D</b>          |             | 1.50 (1.59) |
| <b>VIT.E</b>          |             | 6.7 (3.5)   |
| <b>DIETARY FIBER</b>  |             | 7.6 (5.4)   |

|                                         |           |
|-----------------------------------------|-----------|
| <b>OLIGOSACCHARIDES</b>                 | 38 (19)   |
| <b>Persistence of disorder</b>          |           |
| Developed a new disorder at T1          | 10 (9.6%) |
| All disorders at T0 disappeared at T1   | 47 (45%)  |
| The disorder(s) persisted at T1         | 8 (7.7%)  |
| No disorder at T0 and no disorder at T1 | 39 (38%)  |

Data are presented as Frequency (Percentage) for categorical variables, and as Mean (SD) for continuous variables.

| <b>Differences in nutrients between subjects in category “Natural diet” (N=55), and those in category “Non-natural diet” (N=49)</b> |                                 |                                     |                |
|-------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|-------------------------------------|----------------|
| <b>Variable</b>                                                                                                                     | <b>Diet natural,<br/>N = 55</b> | <b>Diet<br/>non natural, N = 49</b> | <b>p-value</b> |
| <b>KCAL</b>                                                                                                                         | 1,417 (338)                     | 1,376 (367)                         | 0.558          |
| <b>PRO</b>                                                                                                                          | 52 (14)                         | 50 (15)                             | 0.509          |
| <b>FAT</b>                                                                                                                          | 63 (19)                         | 59 (20)                             | 0.324          |
| <b>CHO</b>                                                                                                                          | 189 (137)                       | 167 (51)                            | 0.277          |
| <b>STARCH</b>                                                                                                                       | 41 (33)                         | 33 (33)                             | 0.250          |
| <b>CHOL</b>                                                                                                                         | 135 (60)                        | 155 (109)                           | 0.251          |
| <b>SATURATED F.A.</b>                                                                                                               | 15.1 (7.0)                      | 13.7 (6.0)                          | 0.277          |
| <b>POLY. F.A.</b>                                                                                                                   | 4.99 (1.83)                     | 4.02 (2.02)                         | 0.012          |
| <b>MONO. F.A.</b>                                                                                                                   | 25 (10)                         | 22 (11)                             | 0.089          |
| <b>CA</b>                                                                                                                           | 412 (190)                       | 421 (219)                           | 0.819          |
| <b>NA</b>                                                                                                                           | 881 (565)                       | 780 (669)                           | 0.410          |
| <b>K</b>                                                                                                                            | 1,386 (592)                     | 1,300 (550)                         | 0.443          |
| <b>P</b>                                                                                                                            | 672 (229)                       | 653 (259)                           | 0.685          |
| <b>FE</b>                                                                                                                           | 4.68 (2.70)                     | 4.47 (2.29)                         | 0.664          |
| <b>ZIN</b>                                                                                                                          | 5.36 (2.38)                     | 5.04 (2.16)                         | 0.482          |
| <b>FOLIC A.</b>                                                                                                                     | 100 (74)                        | 104 (75)                            | 0.793          |
| <b>NIACIN</b>                                                                                                                       | 8.2 (4.4)                       | 7.5 (4.2)                           | 0.433          |
| <b>RIBOFLAVIN</b>                                                                                                                   | 0.82 (0.33)                     | 0.90 (0.64)                         | 0.466          |
| <b>THIAMINE</b>                                                                                                                     | 0.53 (0.23)                     | 0.49 (0.21)                         | 0.373          |
| <b>VIT.A</b>                                                                                                                        | 333 (201)                       | 382 (214)                           | 0.234          |
| <b>VIT.B6</b>                                                                                                                       | 0.87 (0.51)                     | 0.82 (0.48)                         | 0.577          |
| <b>VIT.C</b>                                                                                                                        | 71 (87)                         | 57 (49)                             | 0.341          |
| <b>VIT.D</b>                                                                                                                        | 1.53 (1.73)                     | 1.47 (1.43)                         | 0.826          |
| <b>VIT.E</b>                                                                                                                        | 7.1 (3.2)                       | 6.4 (3.8)                           | 0.308          |
| <b>DIETARY FIBER</b>                                                                                                                | 8.1 (5.5)                       | 7.1 (5.2)                           | 0.382          |
| <b>OLIGOSACCHARIDES</b>                                                                                                             | 40 (20)                         | 34 (17)                             | 0.119          |

P-values were computed with Student’s t test or Mann-Whitney U as appropriate. Significant p-values were marked in bold.

| <b>Differences in nutrients between subjects in category “All disorders at T0 disappeared at T1” (N=47), and those in category “The disorder(s) persisted at T1” (N=8)</b> |          |           |               |                |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|-----------|---------------|----------------|
| <b>GROUP DIET A</b>                                                                                                                                                        |          |           |               |                |
| <b>Characteristic</b>                                                                                                                                                      | <b>N</b> | <b>OR</b> | <b>95% CI</b> | <b>p-value</b> |
| <b>KCAL</b>                                                                                                                                                                | 30       | 1.00      | 0.99, 1.00    | 0.203          |
| <b>PRO</b>                                                                                                                                                                 | 30       | 0.98      | 0.90, 1.05    | 0.621          |
| <b>FAT</b>                                                                                                                                                                 | 30       | 0.96      | 0.89, 1.02    | 0.180          |

|                  |    |      |            |       |
|------------------|----|------|------------|-------|
| CHO              | 30 | 0.99 | 0.96, 1.01 | 0.310 |
| STARCH           | 30 | 1.00 | 0.97, 1.03 | 0.989 |
| CHOL             | 30 | 1.00 | 0.97, 1.01 | 0.638 |
| SATURATED F.A.   | 30 | 0.92 | 0.71, 1.09 | 0.396 |
| POLY. F.A.       | 30 | 1.17 | 0.66, 2.06 | 0.571 |
| MONO. F.A.       | 30 | 1.01 | 0.91, 1.12 | 0.887 |
| CA               | 30 | 1.00 | 0.99, 1.01 | 0.995 |
| NA               | 30 | 1.00 | 1.00, 1.00 | 0.822 |
| K                | 30 | 1.00 | 1.00, 1.00 | 0.970 |
| P                | 30 | 1.00 | 1.00, 1.01 | 0.423 |
| FE               | 30 | 1.12 | 0.81, 1.50 | 0.451 |
| ZIN              | 30 | 0.89 | 0.52, 1.35 | 0.588 |
| FOLIC A.         | 30 | 1.00 | 0.99, 1.01 | 0.817 |
| NIACIN           | 30 | 1.06 | 0.85, 1.29 | 0.564 |
| RIBOFLAVIN       | 30 | 3.77 | 0.21, 65.4 | 0.345 |
| THIAMINE         | 30 | 3.48 | 0.04, 225  | 0.550 |
| VIT.A            | 30 | 1.00 | 0.99, 1.00 | 0.650 |
| VIT.B6           | 30 | 1.74 | 0.28, 8.65 | 0.514 |
| VIT.C            | 30 | 0.99 | 0.97, 1.01 | 0.421 |
| VIT.D            | 30 | 0.93 | 0.34, 1.47 | 0.816 |
| VIT.E            | 30 | 1.00 | 0.72, 1.38 | 0.999 |
| DIETARY FIBER    | 27 | 1.10 | 0.89, 1.37 | 0.358 |
| OLIGOSACCHARIDES | 30 | 1.00 | 0.94, 1.05 | 0.994 |

**Differences in nutrients between subjects in category “All disorders at T0 disappeared at T1” (N=47), and those in category “The disorder(s) persisted at T1” (N=8)**

| GROUP DIET B   |    |      |            |         |
|----------------|----|------|------------|---------|
| Characteristic | N  | OR   | 95% CI     | p-value |
| KCAL           | 25 | 0.99 | 0.99, 1.00 | 0.055   |
| PRO            | 25 | 0.97 | 0.87, 1.06 | 0.461   |
| FAT            | 25 | 0.83 | 0.65, 0.95 | 0.003   |
| CHO            | 25 | 0.99 | 0.96, 1.02 | 0.653   |
| STARCH         | 25 | 0.99 | 0.94, 1.03 | 0.757   |
| CHOL           | 25 | 0.99 | 0.97, 1.00 | 0.236   |
| SATURATED F.A. | 25 | 0.88 | 0.68, 1.07 | 0.210   |
| POLY. F.A.     | 25 | 0.30 | 0.04, 0.94 | 0.035   |
| MONO. F.A.     | 25 | 0.77 | 0.52, 0.95 | 0.006   |
| CA             | 25 | 1.00 | 0.99, 1.00 | 0.433   |
| NA             | 25 | 1.00 | 0.99, 1.00 | 0.364   |
| K              | 25 | 1.00 | 1.00, 1.00 | 0.778   |
| P              | 25 | 1.00 | 0.99, 1.00 | 0.276   |
| FE             | 25 | 0.65 | 0.26, 1.21 | 0.199   |
| ZIN            | 25 | 0.42 | 0.12, 1.04 | 0.063   |
| FOLIC A.       | 25 | 1.00 | 0.98, 1.02 | 0.833   |
| NIACIN         | 25 | 0.97 | 0.64, 1.41 | 0.876   |
| RIBOFLAVIN     | 25 | 0.39 | 0.01, 2.17 | 0.412   |
| THIAMINE       | 25 | 0.13 | 0.00, 122  | 0.567   |
| VIT.A          | 25 | 1.00 | 0.99, 1.01 | 0.988   |
| VIT.B6         | 25 | 0.86 | 0.02, 27.9 | 0.930   |
| VIT.C          | 25 | 1.01 | 0.99, 1.03 | 0.373   |
| VIT.D          | 25 | 0.58 | 0.07, 2.11 | 0.464   |

|                         |    |      |            |       |
|-------------------------|----|------|------------|-------|
| <b>VIT.E</b>            | 25 | 0.51 | 0.18, 0.91 | 0.017 |
| <b>DIETARY FIBER</b>    | 20 | 0.95 | 0.66, 1.25 | 0.728 |
| <b>OLIGOSACCHARIDES</b> | 25 | 1.01 | 0.94, 1.08 | 0.753 |

**Differences in nutrients between subjects in category “All disorders at T0 disappeared at T1” (N=47), and those in category “The disorder(s) persisted at T1” (N=8)**

| <b>GROUPS DIET A - B</b> |          |           |               |                |
|--------------------------|----------|-----------|---------------|----------------|
| <b>Characteristic</b>    | <b>N</b> | <b>OR</b> | <b>95% CI</b> | <b>p-value</b> |
| <b>DIETA</b>             | 55       |           |               | 0.780          |
| <b>Naturelle</b>         |          | —         | —             |                |
| <b>Non naturelle</b>     |          | 1.24      | 0.26, 5.81    |                |
| <b>KCAL</b>              | 55       | 0.99      | 0.98, 0.99    | <b>0.034</b>   |
| <b>PRO</b>               | 55       | 0.98      | 0.92, 1.03    | 0.388          |
| <b>FAT</b>               | 55       | 0.93      | 0.87, 0.98    | <b>0.006</b>   |
| <b>CHO</b>               | 55       | 0.99      | 0.97, 1.01    | 0.271          |
| <b>STARCH</b>            | 55       | 1.00      | 0.97, 1.02    | 0.795          |
| <b>CHOL</b>              | 55       | 0.99      | 0.98, 1.00    | 0.252          |
| <b>SATURATED F.A.</b>    | 55       | 0.90      | 0.76, 1.03    | 0.131          |
| <b>POLY. F.A.</b>        | 55       | 0.86      | 0.55, 1.25    | 0.441          |
| <b>MONO. F.A.</b>        | 55       | 0.95      | 0.87, 1.02    | 0.133          |
| <b>CA</b>                | 55       | 1.00      | 0.99, 1.00    | 0.540          |
| <b>NA</b>                | 55       | 1.00      | 1.00, 1.00    | 0.778          |
| <b>K</b>                 | 55       | 1.00      | 1.00, 1.00    | 0.824          |
| <b>P</b>                 | 55       | 1.00      | 1.00, 1.00    | 0.896          |
| <b>FE</b>                | 55       | 1.00      | 0.74, 1.28    | 0.980          |
| <b>ZIN</b>               | 55       | 0.75      | 0.46, 1.10    | 0.160          |
| <b>FOLIC A.</b>          | 55       | 1.00      | 0.99, 1.01    | 0.793          |
| <b>NIACIN</b>            | 55       | 1.03      | 0.85, 1.21    | 0.719          |
| <b>RIBOFLAVIN</b>        | 55       | 0.89      | 0.14, 2.66    | 0.864          |
| <b>THIAMINE</b>          | 55       | 1.35      | 0.03, 37.7    | 0.866          |
| <b>VIT.A</b>             | 55       | 1.00      | 1.00, 1.00    | 0.790          |
| <b>VIT.B6</b>            | 55       | 1.42      | 0.28, 5.48    | 0.638          |
| <b>VIT.C</b>             | 55       | 1.00      | 0.98, 1.01    | 0.872          |
| <b>VIT.D</b>             | 55       | 0.84      | 0.34, 1.35    | 0.576          |
| <b>VIT.E</b>             | 55       | 0.85      | 0.64, 1.06    | 0.155          |
| <b>DIETARY FIBER</b>     | 47       | 1.02      | 0.87, 1.18    | 0.762          |
| <b>OLIGOSACCHARIDES</b>  | 55       | 1.00      | 0.96, 1.04    | 0.910          |

P-values were computed with univariate logistic regression. Significant p-values were marked in bold.

| <b>Group A</b>       |                   |                    |                |
|----------------------|-------------------|--------------------|----------------|
| <b>All disorders</b> | <b>T1 Absence</b> | <b>T1 Presence</b> | <b>p-value</b> |
| <b>T0 Absence</b>    | 19                | 6                  | <0.001*        |
| <b>T0 Presence</b>   | 26                | 4                  |                |

P-values are computed with Mc Nemar test.

| <b>Group B</b> |  |  |  |
|----------------|--|--|--|
|----------------|--|--|--|

| All disorders | T1 Absence | T1 Presence | p-value |
|---------------|------------|-------------|---------|
| T0 Absence    | 20         | 4           | 0.001*  |
| T0 Presence   | 21         | 4           |         |

P-values are computed with Mc Nemar test.

Second, the study found that the prevalency of FGIDs could be related to the quantitative intake and the percentage of fat in the diet. For children with celiac disease, how to rationalize the quantitative intake? Does the author have any appropriate suggestions?

Response 2: Thank you for your comment. As suggested we further speculated in the discussion as follow. "Our study found that the prevalence of FGIDs could be related to the quantitative intake and the percentage of fat in the diet. For children with celiac disease, the best way to rationalize the quantitative intake of fat consumed in the diet is to rely on an expert nutritionist who can recommend a diet with a preference for healthy products that are naturally gluten-free and well-balanced macronutrients"

#### Response to Reviewer 2 Comments

We thank the reviewer for the constructive and positive comments and suggestions and we herein provide a point-to-point response

Very nice article dealing with functional gastrointestinal disorders, celiac disease and gluten-free diet. I just want to recommend to authors some changes to improve their manuscript before publication.

Abstract: please write numbers of patients in methods and not in results.

Response 1: Thanks for your comment. We mooved the numbers of patients from the results to methods section

"Methods.. We prospectively enrolled 104 consecutive CD children newly diagnosed, 55 patients in Group A (53%) and 49 patients in Group B (47%).

Introduction: is true, that typical CD symptoms, but “asymptomatic” fall under atypical forms. This is not exactly pronounced. It is also giving question if FGIDs are in relationships with atypical forms and this can be more explained in discussion.

Response 2: Thanks for your comment. We differentiated the typical form from the atypical one in the introduction and used this point to broaden the discussion

“Introduction

CD is now recognized as a global disease with a prevalence of about 1% of the world's population (2). The clinical presentation ranges from features of malabsorption such as abdominal pain, diarrhea, steatorrhea, and weight loss or growth failure, to atypical forms of celiac disease with more subtle gastrointestinal manifestations similar to functional gastrointestinal disorders (FGIDs) or asymptomatic individuals diagnosed by screening high-risk groups (3). ”

“Discussion

((Patients with CD and FGIDs could somehow fall under the “atypical forms” of celiac disease although, usually, the term "atypical" celiac disease is used for patients who present with extraintestinal symptoms like Immunoglobulin A (IgA)-nephropathy, hemosiderosis of the lungs and a variety of neurological diseases while for FGIDs the symptoms are mainly gastrointestinal. Moreover, another difference is that the atypical forms generally respond to the gluten-free diet with the disappearance of symptoms. (Holtmeier W, Caspary WF. Celiac disease. Orphanet J Rare Dis. 2006 Mar 1;1:3. doi: 10.1186/1750-1172-1-3.)”

Results: please do not repeat information from methodology.

Response 3: Thanks for your remark. We delete from the results the repetitive information

Discussion: please discuss and some sentences are for conclusion. Other, is nice study and after these changed can be published.

Response 3: We thank the reviewer for his/her comments and for the opportunity to improve our manuscript. We modified the discussion separating it from the a conclusion : “

In conclusion, this is the first study to show that the presence of functional GI symptoms in children with CD on a GFD are possibly related to higher caloric and fat intake. Whether the risk is due to the residua of a chronic inflammatory process, and/or due to dietary factors remains to be further tested. Longer term follow-up studies will help determine the natural history of these functional symptoms.”

Reviewer 3

This is an excellent study that suggest a possible correlation between a higher caloric and fat intake and the presence of functional GI symptoms in children with CD on a GFD. This study provide some answer for the residual refractory symptoms in treated coeliac patients. It is very well known that a high calorie in particular a high fat content is immunogenic and associated with inflammatory disorders. This is why a low fat diet is an essential part of Nutrition therapy in Crohn's disease as well. The study is well designed and beautifully written. I cant think of any additional limitation more than what the authors highlighted in the manuscript

We thank the Reviewer to his/her support to our publication.