Author’s response to reviews

Title: Are self-reported gastrointestinal symptoms among older adults associated with increased intestinal permeability and psychological distress?

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Version: 1 Date: 28 Feb 2018

Author’s response to reviews:

Dear Professor Cameron,

Thank you for giving us the opportunity to improve our manuscript. We hereby submit a revised version of our manuscript BGTC-17-00341 after having carefully considered the reviewers' comments and suggestions.

We have addressed the reviewers' suggestions and revised the manuscript accordingly. See point-by-point response below. All changes are marked in yellow.
We hope that you now will find our manuscript suitable for publication in BMC Geriatrics and are looking forward to your reply.

Sincerely,

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Point-by-point response to reviewers:

Reviewer 1 (Inna Lisko)

1 [Heading]: In the heading it is stated that "Self-reported gastrointestinal symptoms among older adults are associated with increased intestinal permeability". Yet, it is clear that zonulin is an indirect indicator of small intestinal permeability. To avoid making a definitive conclusion of zonulin in the heading, the heading could be changed to a question form: "Are self-reported gastrointestinal symptoms among older adults associated with increased intestinal permeability and psychological distress?"

Reply: We agree with the Reviewer and the heading have now been changed in accordance with the proposed suggestions; "Are self-reported gastrointestinal symptoms among older adults associated with increased intestinal permeability and psychological distress?"
2 [Introduction]: Please report and provide a reference on how common in percentages gastrointestinal symptoms are among older adults.

Reply: We thank the Reviewer for pointing out the need for clarification. The following sentence have been added together with references "Fifty to seventy % of older adults report symptoms of constipation (Bosshard et al 2004, Kinnunen et al 1991) and 4-14% experience problems with diarrhoea (Scallan et al 2005, Talley et al 1992, Pilotto et al 2008)", page 4, line 58-61.

3 [Introduction]: Along with gastrointestinal symptoms, cognitive decline is mentioned to be common among older adults (page 4, line 58). This is true but it seems odd to only mention cognitive decline.

Reply: We agree with the Reviewer and "cognitive decline" has now been removed. The sentence now reads: "Gastrointestinal symptoms are common among older adults" (page 4, line 58).

4 [Introduction]: On page 4, line 59, it is stated: "Diseases of the digestive system are known to contribute to a higher symptom burden [3]." Yet, these results are based on a cross-sectional setting. Thus, it cannot be known whether or not diseases of the digestive system exactly "contribute" to a higher symptom burden. Please revise the text.

Reply: We thank the Reviewer for pointing the need for clarification, the sentence has been revised to: "In addition, older adults suffering from diseases of the digestive system are at risk of a higher total symptom burden" page 4, line 59-61.
5 [Introduction]: On page 4, line 66, please provide a reference to the following statement: "Increased intestinal permeability is a hallmark of many GI diseases".

Reply: We thank the Reviewer for noticing the missing references. We have included the following three references 1) Vasoactive Intestinal Polypeptide and Mast Cells Regulate Increased Passage of Colonic Bacteria in Patients With Irritable Bowel Syndrome, Keita et al, 2017, Gastroenterology, 2) Importance of disrupted intestinal barrier in inflammatory bowel diseases, Söderholm et al, 2011, Inflammatory Bowel Diseases 3) The intestinal barrier: a fundamental role in health and disease, Vermeire et al, 2017, Expert review of gastroenterology & hepatology, to support the statement that "increased intestinal permeability is a hallmark of many GI diseases", p4, line 68.

6. [Introduction]. Please provide the basic information on the physiology behind disrupted barrier function and explain what it means.

Reply: A short section describing the physiology behind a disrupted barrier function has now been added, page 4, lines 69-73.

"The intestinal epithelium is the major interface with the external environment and while absorbing nutrients and water it simultaneously restricts the free movement of luminal material to the underlying mucosa (Bischoff et al 2014). A disruption of the intestinal barrier may result in the passage of microbial antigens and toxins (Suzuki 2013), and are associated with intestinal inflammation as well as neurological diseases".

7. [Introduction]. On page 4, line 70, I believe the word "character" is supposed to be "characteristics".
8. [Introduction]. Please provide already in the introduction a reference for zonulin (page 4, line 73).

Reply: We have added the following references: 1) Human zonulin, a potential modulator of intestinal tight junctions, Fasano et al, 2000, Journal of cell science 2) Zonulin upregulation is associated with increased gut permeability in subjects with type 1 diabetes and their relatives, Fasano et al, 2006, Diabetes, in the introduction where zonulin is mentioned, p 5, line 78.

9. [Methods]. p.6, line 118. Please provide also in the methods section a reference which states that zonulin is an indirect indicator of small intestinal permeability.

Reply: The following reference has been added "Zonulin upregulation is associated with increased gut permeability in subjects with type 1 diabetes and their relatives" by Fasano et al, 2006 in page 6, line 117.

10 [Results]. When reporting the results on psychological distress, the following is stated (page 8, line 148): "Assessment of overall psychological distress (total HADS score), showed increased levels among older adults with GI symptoms compared to general older adults (Table 4)." However, this does not seem to be the case. According to Table 4 there were no statistically significant differences in assessment of overall psychological distress between older adults with GI symptoms and general older adults. Furthermore, the last sentence in the paragraph (page 8, line 154) deals with the same results, and it would be easier for the reader if all results on total HADS score were reported in same place. Please revise the text.
Reply: We thank the Reviewer for pointing out the need for clarification. The significant differences are observed between senior orienteering athletes and the two other groups of older adults, not between general older adults and older adults with GI symptoms. The sentence has been revised to "Assessment of overall psychological distress (total HADS score) showed increased levels for both older adults with GI symptoms (p<0.05) and general older adults (p<0.05) when compared to the senior orienteering athletes (Table 1).", page 8, lines 153-155. We further deleted the last paragraph as the text was redundant.

11 [Results]. In the paragraph for comorbidity and medications, compared to which group multipharmacy was more prominent than in older adults with GI symptoms (page 9, line 172)? Please specify.

Reply: We have revised this section and the following sentence is now included "Multipharmacy (defined as intake of five or more medications simultaneously) was found to be more prominent in older adults suffering from GI symptoms (12.5%) compared to both senior orienteering athletes (3.7%) and general older adults (0%)", page 9, lines 179-183.

12 [Results]. If there is a reference (references) available on the subsequent study results showing that medication and comorbidities did not influence zonulin levels or psychological distress, please provide it (page 9, lines 173-175).

Reply: We thank the Reviewer for pointing out the misunderstanding regarding our results and need for clarification. The subsequent analysis mentioned is a part of the present study and aim to explain to the reader that confounding effects of medication and comorbidities was controlled for, hence, there is no reference available. To clarify that the analysis mentioned is a part of the present study we have now revised the sentence to: The levels of zonulin and psychological
distress was further found not be influenced by medication and comorbidities when confounding effects were controlled for, p 9, line 181-183.

13 [Table 1]. Please report also the body mass index (BMI) of the participants if available.

Reply: We agree with the Reviewer that BMI data would have been an asset and would add important information to the manuscript. Unfortunately, BMI data are only available for elderly with GI symptoms and general older adults, but are lacking for the senior orienteering athletes. Hence, we choose not to include BMI data in table 1. Instead we have included a section in the discussion where we discuss other sources for circulating zonulin according to the reviewers point nr 20, p 14, line 264-268.

14 [Table 1]. The marking on the GSRS diarrhoea/constipation score is unclear. Please revise.

Reply: We thank the Reviewer for noticing the misplacing of ≥, we have now corrected this and the inclusion criteria for older adults with GI symptoms now reads "GSRS diarrhoea/constipation score ≥ 3" (marked in yellow). In addition we removed the marking III as a section regarding the rationale behind choosing constipation and/or diarrhoea as inclusion criteria is included in the methods section.

15 [Table 1]. Please inform what the acronym GSRS stands for.

Reply: We have added this important information according to the Reviewers comment. A * has been added at the end of GSRS and FGAS and the abbreviations used in the table are explained under Table 1.
16 [Table 3]. The marking on the grading of GSRS is not clear (">") should not be there. Please revise.

Reply: We are grateful the Reviewer noticed this mistake. We have removed the symbol ">" from Table 3.

17 [Table 4]. Please inform what the acronym HADS stands for.

Reply: We thank the Reviewer for noticing the need for clarification. However, according to the comment made by Reviewer number 3, regarding redundancy between table and figures, we have now moved the information regarding HADS total score to table 1 and thereby table 4 does no longer exit in the manuscript.

18 [Discussion]. The authors state (page 13, line 226): "Experience of anxiety did not differ between older adults with and without GI symptoms, suggesting that anxiety increases with age." The suggestion "anxiety increases with age" cannot be made based on these results. Please revise.

Reply: We agree with the Reviewer and the sentence has now been revised to the following: "Experience of anxiety did not differ between older adults with and without GI symptoms." The last part of the sentence has been removed. Page 12-13, line 226-227.

19 [Discussion]. It could be pointed out what is considered the gold standard to assess intestinal permeability.
Reply: We thank the Reviewer for pointing out this and we have now added the following sentence at page 14-15 lines 274-278.

"Nevertheless, the findings presented here needs to be confirmed in future studies using more advanced techniques, such as the Ussing Chamber methodology. This will allow for a thorough assessment ex vivo of the intestinal barrier function in elderly using mucosal biopsies (Ganda mall et al 2017, Keita et al 2017, Keita et al 2006) and hence add important information to the results presented here".

20 [Discussion]. Literature shows that zonulin is also associated with BMI (Moreno-Navarrete et al. 2012. Circulating zonulin, a marker of intestinal permeability, is increased in association with obesity-associated insulin resistance. PLoS One. 2012; 7(5):e37160). BMI should be included in the paper if available, and the results should be discussed.

Reply: We thank the Reviewer for this important observation. We fully agree that BMI data would add an important perspective. Unfortunately, BMI data on the senior orienteering athletes are not available as mentioned above in response to point nr 13. However, analysis of existing BMI data for older adults with GI symptoms and general older adults did show that the data were normally distributed in the two study groups and no significant differences were identified. Thus indicating that the zonulin levels were not affected by BMI. However, we agree with the Reviewer that this is important to include in the manuscript. As the data for the senior orienteering athletes are not available and could not be included in a table we choose to discuss the importance of BMI and the following section has been added "However, the body mass index (BMI) of the general older adults (25.6±4.3 Std) and older adults with GI symptoms (26.7±5.0 Std) included in the present study was normally distributed and no significant differences were observed between the two groups. In addition, stratification of the data revealed no association between cardiovascular disease, including hypertension, and increased zonulin levels.", page 14, lines 268-274.
Please discuss the role of nutrition in intestinal permeability.

Reply: This is an important and highly relevant area. Particularly, fibre intake is often discussed in relation to elderly as this part of the population is known to consume a low intake of fibres. Hence, we have included the following section. "Moreover, elderly individuals are known to have a low fibre intake (Donini et al 2009) that in addition to a disturbed intestinal motility could altered the gut microbiota and result in a diminished diversity that could have a negative impact on the intestinal barrier function (Kelly et al 2015). Recently, we showed that a dietary fibre from yeast was able to attenuate stress-induced hyperpermeability ex vivo across small intestinal tissue from Crohn's disease patients mounted in the Ussing Chamber (Ganda Mall et al 2017). Thus, dietary fibres could be a potential therapeutic able to strengthen the intestinal barrier in elderly individuals, however, this needs to be thoroughly investigated in pre-clinical and clinical settings", p 14, line 253-259.

22 [Discussion]. On page 14, line 254 the sentence "Moreover, the median plasma zonulin…" should be placed right after the previous sentence "However, only three participants reported…” (page 14, line 253).

Reply: This has now been adjusted according to the Reviewers' suggestion, page 15, line 285-287.

Reviewer 2 (Gerard Clarke)

1: The authors are careful in the manuscript to indicate that zonulin is an indirect marker of intestinal permeability. However, there is still some debate regarding the precise meaning of circulating zonulin since it may also be derived from sources other than the gut (e.g. see paper
from Ohlsson et al., 2017 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5372598/#B2-ijms-18-00582). The discussion would therefore benefit from including this and other caveats surrounding this marker, particularly in the context of the increased cardiovascular disease in the older adults with GI symptoms. Can the authors also include the limit of detection and quantification and the intra-assay variability for the kit they used?

Reply: We thank the Reviewer for this valuable comment and as zonulin has been, and is, under debate as a marker of intestinal permeability it is important to discuss, we have added the following section in the discussion "However, zonulin as a marker of small intestinal permeability has been, and is, under debate. Levels of zonulin have been found to fluctuate over time making interpretation of the results difficult (Vojdani et al 2017). Moreover, a recent study suggests that circulating zonulin might not only be derived from the gastrointestinal tract but may be associated with obesity and hyperlipidemia (Ohlsson et al 2017, Moreno-Navarrete et al 2012). However, the body mass index (BMI) of the general older adults (25.6±4.3 Std) and older adults with GI symptoms (26.7±5.0 Std) included in the present study was normally distributed and no significant differences were observed between the two groups. In addition, stratification of the data revealed no association between cardiovascular disease, including hypertension, and increased zonulin levels. Even though BMI was not available for the senior orienteering athletes, these findings indicate that the increased zonulin levels in the present study were not a result of overweight/obesity or cardiovascular disease", p 14, line 268-274.

Furthermore, we have included the limit of detection and quantification and the intra-assay variability for the kit used, p 7, line 124-126.

2: If we accept the bonafides of zonulin as a readout for intestinal permeability, the fact that CRP levels are not elevated despite increased zonulin levels may indicate that the alteration noted is not sufficient to induce the immune activation necessary to back up aspects of the rationale for the study. If there are no inflammatory consequences of increased intestinal permeability, is it a
valid therapeutic target or are we just seeing the normal physiological range of intestinal permeability in old age? As the authors themselves note, cytokine levels would be beneficial here and add value to the study. Would it be possible to add this analysis to the current study?

Reply: We agree with the reviewer that this would be necessary to include in order to make an accurate conclusion of the findings. Unfortunately, we do not have the possibility to include these analyses in the present manuscript as we do not have blood samples or intestinal biopsies available. However, a study is currently conducted to follow up the results in the present manuscript and we will take the Reviewer's comment with us when preparing the future manuscript.

3: The authors have also been careful to link the HADS score to psychological distress apart from one instance in the results section on page 8 where they suggest that depression is higher in older adults with GI symptoms. This should be changed to indicate probable depression or alternative preferred description in line with the limited capacity of the HADS as a diagnostic tool for depression.

Reply: We have now revised this section and added "probable depression" according to the Reviewers' suggestion, p 8, line 155. In addition, we changed the wording in the discussion to depression-like characteristics, p 12, line 222.

Reviewer 3 (Melanie Gareau)

1: The authors need to state and reference the cut off point for the HADS used to determine the levels of anxiety and depression in the participants. Although the reported levels of both HADS depression/ anxiety scores are statistically significant between the groups they seem to be all below the cut-off point for a typical HADS score (8/21), Bjelland, I; et al. (2002). This needs to be discussed.
Reply: We thank the Reviewer for pointing out the need for clarification. We have added the following section in the discussion "However, the levels of psychological distress estimated by HADS were below the cut-off value (≥ 8) for severe anxiety and depression. Hence, indicating that none of the study participants suffered from severe psychological disease", p 13, line 228-231. Furthermore, we have changed the wording in the discussion to depression-like characteristics instead of depression to emphasize that the scores do not reflect clinical depression.

2: In the current version, much of the data seems duplicated in figure and table form, making it seem redundant.

Reply: We thank the Reviewer for noticing the duplication in information. We have now moved the information regarding HADS total scores presented in table 4 to table 1 making table 4 redundant which was therefore deleted.

3: The GI distress group has significantly more females than males, which is in contrast to the control groups. This discrepancy was not addressed in the manuscript, but could significantly impact results and conclusions. This needs to be discussed.

Reply: We agree with the Reviewer that this needs to be discussed. We have now addressed this comment and added the following section in the discussion "Females (n=18) were further overrepresented among elderly suffering from GI symptoms (n=24). This could reflect the normal distribution of GI symptoms in the population as previous studies report an increased frequency of GI symptoms, such as constipation, among women (Walter et al 2002, Higgins et al 2004, Wald et al 2008). Stratification for gender did not reveal a significant difference between men and women in regard to GI symptoms, zonulin levels and depression-like characteristics. Anxiety-like characteristics were, on the contrary, found to be significantly higher among elderly
men with GI symptoms (n=6). However, this finding could be due to the small study population and further studies using larger sample sets will need to be performed in order to thoroughly elucidate the difference between men and women in relation to psychological distress, GI symptoms and intestinal barrier function." , p 13, line 237-246.