May 1st, 2018

Dear Ze-Mao Gong,

Science Editor of *World Journal of Gastroenterology*

We thank the editor and reviewers for the opportunity to submit our revised manuscript **(ESPS Manuscript NO:** 39021**)** entitled: "The Mediterranean dietary components are inversely associated with advanced colorectal polyps". The manuscript has been corrected in accordance with the reviewer's comments. All the corrections in the manuscript are **highlighted in yellow**.

Attached is a point-by point reply to the reviewers.

Sincerely,

Prof. Shira Zelber-Sagi
Head of Nutrition, Health and Behavior program
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Reviewer 1: (Reviewer's code: 00033377)

1. Results: 1) Should include p-values on Table 1

   Answer: P-values were added (Table 1).

2. Methods: 1) Need a definition of what constitutes an alarming symptom

Answer: Thank you, the alarming symptoms have been specified (Page 8)

3. Discussion: 1) I would compare a bit more this study results with prior literature on Mediterranean diet and colorectal adenomas. How is this study different?

   Answer: A greater emphasis has been made on discussing the association between the MD score and colorectal neoplasia in the discussion (Page 14-15), with indication of the differences between this study results and previous studies.

4. 2) On page 14, it is mentioned that consumption of fruit and fish and low sugar were independently associated with advanced polyps after adjusting for medical history. Yet fig 2 does not seem to adjust for medical history.

   Answer: In figure 2 associations presented were adjusted for family history of CRC, personal indication for colonoscopy and use of medications, together presented as medical history. Following the comment by Reviewer 3, Figure 2 has been removed from the analysis.

5. 3) I believe a significant limitation is the inclusion of patients with alarming symptoms as opposed to having included patients for screening and surveillance only. Even tough groups were evenly matched for alarming symptoms, it is unclear what is an alarming symptom and likely not all of these carry the same weight to predict colon polyps.

Answer: As mentioned above, alarming symptoms have been specified and did not differ between cases and controls. We believe this inclusion did not significantly affect our results as these are not necessarily associated with hereditary tendency for polyps or cancer. In a sensitivity analysis added to the manuscript, we present an analysis excluding cases of surveillance colonoscopies (Page 13, supplementary figure), as this indication was a feature of only the colorectal polyp cases and not the controls (by definition). The sensitivity analysis results support the main analysis.

Reviewer 2: (Reviewer's code: 01555255)

1. Introduction section: On the basis of its components, the literature reports on the effectiveness of the Mediterranean diet in reducing cardiovascular risk and in preventing major chronic diseases, including obesity and diabetes (e.g. Abenavoli et al. World J Gastroenterol 2014). I suggest also to include the prevalence of CRC in Israel.

   Answer: Details of CRC incidence worldwide and in Israel have been added to the introduction. Also, we elaborated on the association of the MD and risk of major chronic diseases including cancer, emphasizing the diet's nutritional properties and potential mechanisms of action (Page 7).

2. Methods section: The cases are evaluated by the same pathologist? Recently a number of studies using laboratory animal models and different cell lines, suggest a possible anti-cancer effects of probiotics (e.g. Kumar et al. Nutr

Cancer 2017). In this context, we have data by the questionnaire on the use of probiotics in the evaluated patients?

Answer: Not all cases were evaluated by the same pathologist unfortunately. However, all were evaluated at the same hospital lab. Also, inter-observer agreement for adenoma is high[1]. Data regarding use of probiotics was not collected in this study.

3. Discussion section: CRC is largely associated with lifestyle factors including diet. Polyphenols are phytochemicals ingested as part of a normal diet, which are abundant in plant foods including fruits/berries and vegetables. These may exert their anti-carcinogenic effects via the modulation of inflammatory pathways. Key signal transduction pathways are fundamental to the association of inflammation and disease progression (e.g. Little et al. Crit Rev Food Sci Nutr. 2017). I sugegst to highlight the role of polyphenols, basic components of Mediterranean diet (e.g. Abenavoli et al. J Transl Int Med. 2017), in the prevention of CRC.

Answer: Thank you for this suggestion. we added more discussion and references on that potential beneficial mechanism of the MD in the introduction and discussion (Page 7 and page 16). With the revision of results according to the recommendations of reviewer 3, performing further mutual adjustments to all other factors of the MD, fruit intake was no longer significantly associated with advanced polyps in a final multivariate analysis. This may be explained by multicollinearity between different food groups of the MD (fruit intake was correlated to vegetable intake for

example) and may have influenced statistical significance of these factors. Still, we present and discussed fruit and its association with advanced polyps with adjustment to all other confounding factors (Table 2, Model 2, Page 16)

Reviewer 3: (Reviewer's code: 00227433)

1. Suggest to add 'a case-control study' to the study title.

   Answer: The study title has been revised as suggested.

2. Abstract – please incorporate that a 116-item FFQ was the dietary assessment tool used.

   Answer: Description of the methods of dietary intake data collection was incorporated in the abstract (Page 4).

3. Introduction – the following sentence should be rephrased or removed as it is too much of a sweeping statement: "It is considered the most evidence-based diet for prevention of chronic disease"

   Answer: This sentence has been revised to a more moderate statement (Page 7)

4. Introduction – the following sentence 'suggested that MD exerts anti-neoplastic properties' – please expand on this. For example, what were the study designs, and what hallmarks of cancer have been affected by the Mediterranean diet to justify this statement?

Answer: The study designs **were specified and the proposed mechanisms by which the MD may affect cancer risk have been outlined in the introduction (Page 6-7).**

5. Methods - the definition of 'advanced' adenomas is stated to be defined according to the accepted guidelines (references 22 and 23). Please clarify that these are US guidelines, as these do not apply to many parts of the world.
   Answer: This clarification has been added to the text (page 9).

6. Methods/throughout – can you please clarify the definition of serrated adenomas included in this study? Were these sessile serrated adenomas/polyps (or lesions) or is this referring to all serrated polyps, which would include hyperplastic polyps, that were large etc. Please adjust the terminology used throughout the manuscript as appropriate.
   Answer: We thank the reviewer for this valuable correction. As the study population was composed of cases with either adenomatouse polyps or advanced serrated polyps (serrated adenoma (>10mm or with dysplasia), the reference to hyperplastic polyps was deleted from the methods. This clarification was added in the text (Page 9).

7. Methods – description of the MD adherence score – a table would be helpful to understand and complement this section of the methods, and to ensure this is easily replicable in other studies.

Answer: Table 2 has been revised to include the specific cutoffs of each dietary component, as recommended. Therefore, we did not add a separate table presenting this data.

8. Results first sentence – please correct small typo (…2543 preforming colonoscopy…).

   Answer: We apologize for the mistake, it has been corrected (Page 10).

9. Results -Do the authors have information on how many patients were approached but did not consent to take part in the study? The reporting of this response rate is very important for understanding how generalisable these patients who were included are compared to the overall patient group attending this hospital. If unknown, please add this as a limitation.

   Answer: Data regarding response rate to participate in the study was added to the results (Page 12), and o the discussion (Page 17).

10. Results/Table 1 – some variables require further explanation and information to interpret in the methods/results text/table footnotes. For example, how was low socio-economic status categorised? The % classified as low is very small, which suggests these results may not be generalisable to lower SES populations. Also how was physical inactivity measured? Are the continuous variables presented as mean/SD or median/range values? Some abbreviations also require explanation in the footnotes.

Answer: We thank the reviewer for this comment, additional information was added to each of the figures and tables of the manuscript. Also, abbreviations were spelled out in the footnotes. We now discuss in more detail a limitation of the study regarding our study sample presenting low proportions of low socio-economic status. We mention that the study population may therefore not be representative of a population of low socio-economic status (Page 17).

11. The population includes a mixture of patients attending for screening, diagnostic or surveillance purposes. Whilst this is acceptable, I would recommend that sensitivity analysis is conducted in which patients who attended for surveillance purposes are removed from analysis or additional stratified analysis in which these patients are separated from screening/diagnostic cases and results still displayed. The surveillance patients' results may be prone to reverse causation bias, which admittedly is likely to result in an underestimate of the odds ratios shown, but is still likely to influence the results. On the other hand, this group may be more likely to be aspirin users, which may have overestimated the results seen. The surveillance group is also more reflective of associations with polyp recurrence, rather than incidence, and so separation of these results would be helpful from that perspective as well.

Answer: A sensitivity analysis, excluding patients undergoing surveillance colonoscopy (n=128) was added to the multivariate analysis of the MD

score (Page 13 and supplementary figure 1). This analysis resulted in estimates similar to those of the main (original) analysis.

12. Table 2 – this is the most important table of results. Overall, I feel there should be some additional results displayed, and the authors may wish to consider splitting this into two tables – one which highlights only the overall Mediterranean diet score results and one which displays the odds ratios for the various components of this score. Either way, the presentation of results should be adapted to show what the results were in analyses that were only adjusted for age/sex, so that the reader can evaluate the impact of confounders on the associations seen, and the reference category should also be more clearly displayed. The values for below and above the median cut-offs should be included in the tables as well. As with table 1, all abbreviations should be explained in the table footnotes.

Answer: We thank the reviewer for this valuable comment, and feel this has greatly improved the presentation of results in this manuscript. The data presented in Table 2 has been divided and is now presented partly in Table 1 (univariate analysis), and in Table 2 (multivariate analysis). Two additional multivariate analysis models are presented – Model 1 and Model 3. In Model 1, each MD component was adjusted for age, gender and BMI as these are all strongly associated with colorectal neoplasia and with diet and are potentially strong confounders for the association. In Model 3 each MD component was adjusted for all confounders in Model 2 and all other

dietary components. Cutoff values are displayed in the table, description of each dietary component and abbreviations were added in the footnotes.

13. Figure 1 is very useful and a good visual display. However, Figure 2 is not as helpful an addition to the paper, since it is selectively reporting only the significant components of the MD score as already shown in Table 2. In addition, the values for OR displayed in Figure 2 do not correspond with Table 2. Please clarify that the ORs in Table 2 are correct, and suggest to remove Figure 2.

    Answer: The OR in Figure 2 stems from a model that further adjusts for the other significant MD components which were not adjusted for in Table 2. Thus, the association and sample sizes differed between Table 3 and Figure 2. We accept the reviewer comment and thus Figure 2 has been removed from the analysis, and the results are now presented in table 2, Model 3. In this model, all dietary components were adjusted for one another, and for all other confounding factors of Model 2.

14. Results/Table 3/Discussion - Similarly to the above comments, unfortunately I do not think the presentation and selective reporting of only the significant results from the overall MD score as currently shown in Table 3 is helpful. This analysis has been split into 16 dietary clusters, which means that the numbers of cases are very small. This seems contradictory to highlight only these components, when the overall Mediterranean diet score should be the main focus of the paper. Likewise, to state in the corresponding discussion

that fish, fruit and sugar-sweetened beverages are independently associated with risk implies that you have mutually adjusted for the other MD components in analysis, which is not the case. I would strongly suggest that this analysis and any corresponding results text is removed from the paper. The overall MD score results should be the main focus, and then it is fine to highlight that this is largely attributed to the most significant components – but to concentrate solely on these four components in further analysis is too selective.

Answer: Table 3 and all related text has been removed from the analysis, results are now concisely presented in table 2, Model 3.

15. Discussion – two previous MD and polyp risk studies are referred to in the discussion (references 2 and 26), one of which is a systematic review. Can further discussion of these studies and comparison/contrasting with the current study results please be incorporated? For example, one is a study of polyp recurrence, rather than incidence. It would also be helpful to highlight these in the introduction.

    Answer: The two studies were added shortly to the introduction and were compared to the current study in more detail in the discussion (Pages 14-15).

16. Discussion – please adapt the discussion to place more emphasis on the overall MD score, and please also acknowledge and discuss the MD components that were not significantly associated with polyp risk, for example alcohol.

Answer: More attention was given to the overall MD score in the discussion (Page 14-15). The non-significant MD components are also discussed (Pages 16-17).

17. Discussion, limitations – these rightfully include 'reporting bias' but can the authors please be more specific about the exact biases here, and include references where possible?

    Answer: Emphasis on the potential report bias of dietary intake assessment have been addressed in the discussion (Page 14). As stated, different strategies were taken to minimize the potential bias.

18. References – reference 5 (the World Cancer Research Fund report) requires some additional information to be included.

    Answer: Thank you, the reference was changed and corrected.

1. Osmond A, Li-Chang H, Kirsch R, Divaris D, Falck V, Liu DF, Marginean C, Newell K, Parfitt J, Rudrick B, Sapp H, Smith S, Walsh J, Wasty F, Driman DK. Interobserver variability in assessing dysplasia and architecture in colorectal adenomas: a multicentre Canadian study. *J Clin Pathol* 2014;**67**:781–6 [PMID: 25004943 DOI: 10.1136/jclinpath-2014-202177]