July 13, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 11898-review.doc).

Title: **Ehealth: Low Fermentable Oligo-Di-Monosaccharides And Polyols versus *Lactobacilus Rhamnosus* GG in Irritable Bowel Syndrome**

Author: Natalia Pedersen, Nynne Nyboe Andersen, Zsuzsanna Végh, Lisbeth Jensen, Dorit Vedel Ankersen, Maria Felding, Mette Hestetun Simonsen, Johan Burisch, Pia Munkholm

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The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer
(1) Review 1. *00159517*

The manuscript addresses an important issue in regards to the influence of diet on IBS symptoms. The authors compare a low FODMAP diet with a probiotic strain against a standard diet. They address the limitations of a non-blinded study which would require further investigation and likely a different study design. The innovative use of an online survey/diary is an interesting approach.

1. A few things that should be considered: careful review of the diction and grammar - some sentences are not clear when describing the population and the process of randomization.

Answer: The diction and grammar in the chapter regarding study population and the process of randomization were corrected. Please see the correction in the manuscript (page 4).

2. In the **discussion**, the authors state that the diet should be followed for at least 6 weeks. Is there any data on maintaining the lower symptom scores if the diet is discontinued after that period? It would be likely that patients may have to stay on the diet for a much longer period of time if not for the rest of their life.

Answer: There is currently no data available indicating a lower symptom score after discontinued diet intervention, however, further studies on this are ongoing at Herlev University Hospital as a follow-up study. This follow up study will indicate whether or not IBS patient have discontinued the diet after the intervention period and the

consequences hereof. In addition, Gibson P and coworkers from Australia are currently investigating the long term (side) effect of the low FODMAP diet in particular the effect of gut microbiota and immunology (Gibson et al. 2013. Inernal Medicine Journal, Muscatello M et al, 2014. WJG). This was discussed, page 11.

All patients that underwent 6 weeks LFD in our study were reintroduced by our dietitians/nutritionists to some of the restricted diet products, however, adjusting the variation and quantity of these products. Afterwards patients were connected to the dietitian/nutritionists for a longer time. This was added to the diet procedures, page 6.

3. Some statements about effectiveness of both LFD and LGG in IBS-D and IBS-A should be revised as this is not supported by the presented data and results section.

Answer: The table about the effectiveness of both LFD and LGG in IBS-D, IBS-A and IBS-C were revised and shown by a table 2 (page 32).

Table 2. Change of the IBS-SSS from baseline and after 6 weeks with LFD vs. LGG vs. ND in different subtypes of IBS patients.

	IBS-D			IBS-A			IBS-C		
	Baseline	6-week	p	Baseline	6-week		Baseline	6-week	p
LFD	320 (110)	153 (136)	<0.01*	359 (66)	241 (111)	0.01*	289 (79)	200 (62)	0.14
LGG	297 (99)	199 (102)	0.01*	251 (64)	187 (122)	0.04*	321 (79)	270 (145)	0.74
ND	320 (89)	257 (118)	0.01*	312 (99)	322 (62)	0.12	302 (70)	277 (135)	0.61

Note: Wilcoxon related samples: Mean IBS-SSS (±SD).
FODMAP: Oligosaccharides, Disaccharides, Monosaccharides And Polyols; LFD: Low FODMAP diet; LGG: *Lactobacillus rhamnosus* GG; ND: normal (Danish/Western) diet; IBS: irritable bowel syndrome; SSS: severity score system; IBS-D: diarrhoea predominant; IBS-C: constipation predominant; IBS-A: alternating periods of diarrhoea and constipation.
* Significant result.

4. Further explanation about the web-based approach should be given in the methods section especially in regards to patient privacy and data protection.

Answer:
The web-application is fully secured based on the net code principles. Each user requires a username and a password in order to access the web-application. Beside the patients, only certain care providers have access to patients' data, allowing continuous monitoring of patients with regards to disease activity if necessary, to avoid complications.
For the research purpose, registered data in the web-database were automatically linked to the Excel export function, allowing statistical analysis. The link between data and the patient was performed via a consequent personal and anonymous patient number, page 6-7 in the Manuscript.

(2)

Review 2. *00008491*

1. GENERAL COMMENT This could be an innovative paper which seems to demonstrate that a self-managed web application could potentially improve the management of the IBS population. However, its major weakness is the lacking of a control group. Perhaps, the Authors could compare the studied population with a historical one, followed for the same period lenght, in which no WEB assistence was (or will be) provided.

Answer: We thank reviewer for the essential comment. However, unfortunately in this study we did not include the historical control group. This limit was added to the study limitations, page 13.

2. Other, about the interventional groups (LFD and conclusions LGG), are very hard to reach. In fact, in these cases also - but in my opinion much more than for the "pure web" group -, the lack of the controls does not permit to reach any conclusions.

Answer. We agree that the major limitation is that the conclusions of the results in interventional groups (LFD and LGG), are very hard to reach, due to lack of respective controls to each group and much more than for the "pure web" group. This was discussed in the limitation (page 13).

3. SPECIFIC POINTS A detailed list of the foods which were avoided by the patients included in the LFD group would be useful. It could be submitted as supplementary file.
   Answer: The lists of the foods which were avoided by the patients in the LFD group are provided. These lists are based on the lists with high FODMAPs from Monash University, Australia, modified and to the Danish population. (Gibson et al 2005. Aliment Pharmacol Ther; Shepherd et al 2008. Clin Gastroenterol Hepathol; Ong et al 2010. Gastroenterology; Gibson et al. 2010. Clin Gastroenterol Hepathol). The original lists are currently much longer than during our study.

Table 1. Supplementary. The list of food not allowed on Low FODMAP diet.

Bread and cereals	**Meat**	**Fruit**	**Vegetables**	**Cake and sweets**
Bran flakes etc. Burger bread Crisp bread Pizza dough Porridge- wheat Rye bread Sausage bread Wheat bread White bread	Lever pâté ** Meat balls**	Apple Banana (green) Blackcurrant Pear Water melon	Artichokes Asparagus Beetroot Beets Broccoli Cabbage* Champignon Corn Fennel Jerusalem artichokes Leek Peas	Licorice contains sugar alcohol sweetener Light products contains sugar alcohol sweetener Whine gum contains sugar alcohol sweetener
Flour	**Dairy products**	**Stone fruits**	**Onions**	**Sugar and sweeteners**
Barley Durum Rye Wheat,incl. whole grain	Blue cheese moldy Cheese spreads Cottage cheese Cream Cream ice Cream fraiche Milk with lactose* Yoghurt Yoghurt beverages White cheese moldy	Avocado Cherry Nectarine Plum	Garlic Onion Spring onions	(Glycol) (Glycerol) Isomalt Lactitol Mannitol Ribitol Sorbitol Sukrin (Erithrytol) Xylitol
Pasta/rice and potatoes	**Fats**	**Beverages**	**Leguminous fruit**	**Dried fruit and nuts**
Bulgur Couscous Pasta of wheat, rye and barley Wheat and barley grain	No restrictions	Dandelion tea Fruit syrup* Juice*	Baked beans Beans* Dhal Falafel Hummus Lentils*	Dried fruits* Pistachio

* All kind

**Meat balls with onions and wheat

**Lever pâté (Danish specialty)

4. The Authors stated that all patients included in the study "had negative outcome of colonoscopy". In this way, I think that it is not useful (also expansive) to perform fecal calprotectin assay. I would suggest taking off this paragraph (page 7).

Answer: The paragraph of fecal calprotectin assay was excluded from the study, page 6 and Table 1.

5. The Authors reported that "eight patients from LFD group drop out due to difficulty with the diet; 8 of 42 patients is 19%: this is a quite high percentage and this should be evaluated in an intention to treat analysis and discussed.

Answer:

We do agree that this should have been analysed and discussed in the paper. The protocol was not designed to have ITT analysis out. If still interest of doing this analysis we can however find the missing data in the patients files out and to do the ITT analysis.

From other studies we would have expected up to 15% drop outs; however, we must admit that the LFD is difficult to follow/adhere to, due to lots of restriction on foods. In addition the feedbacks we have had from the patients are that the LFD is also expensive and requires more time for preparing the daily meals. On the other hand, we did not expect a drop out in the LGG group of 10% which is nearly the same as ND (observational group) 8%. Relative to the LGG group which only had to take some capsules a day, given to them at the hospital without any expenses in relation to this intervention, it makes sense that the drop outs in LFD group is higher than 10%. A 10% difference in drop outs from the ND and LFD is somehow expected due to the fact that the diet is difficult to follow as stated above. One can say that the drop outs is higher in all groups than expected but the relativeness between the groups remains the same and therefore comparative non parametric statistics is right – we have not used parametric statistic to state how much better one group is doing relative to another group.

6. Furthermore, the Authors reported that "There were a higher number of consultations in the LFD group (45 %), mostly due to the questions regarding the diet". This is indicative of the difficult to adhere to this kind of the diet. This difficult should be underlined and the likelihood that this diet can be accepted for a long time should be discussed.

Answer: We agree that higher number of consultations and high percentage of patients that dropped out of the LFD group elucidate the difficulty with this kind of the diet. The difficulty to follow/adhere to LFD in our study was firstly due to lots of restriction on foods. In addition the feedbacks we have had from the patients are that the LFD is also

expensive, difficult to find the products in the stores and requires more time for preparing the daily meals.
The difficulty with the diet was discussed (page 11-12).

(3)
Review 3. *02861597*

Interesting study. 1. The non-blinded nature of the study design is obviously a problem. While I acknowledge the challenges that dietary studies pose, a placebo for the probiotic could have been included.
Answer: We agree with the comment. However, the protocol was not constructed to compare separate groups with the placebo but between the groups, please see the limitations, page 13.

2. How was the study powered?
Answer: The primary end point of this study is IBS-SSS. We cannot based on this end point assume normally distributed data which means that we have to perform non parametric statistics on the primary end point. Therefore power has been set to 80% and type 2 errors to 20% and type 1 error to 5% – see below sample size calculation.

$$n_1 = n_2 \geq \frac{2\left(z_{1-\alpha/2} + z_{1-\beta}\right)^2 * SD^2}{d^2} = \frac{2(1{,}96 + 0{,}84)^2 * 80^2}{50^2} = 40$$

The sample size calculation shows the need of 40 subjects per each treatment group. SD has been found from other studies and d has been estimated as 10% of the scale of the primary end point, page 4.

3. How were corrections for multiple comparisons made?
Answer: "Multiple comparisons (MC) imply of course a type 1 error issue. To this end, we compared the 3 treatment 'arms' on the primary endpoint using a single test based on a non-parametric one-way ANOVA. Since this test showed significant differences, we then compared the different arms pairwise. Thus the MC issue linked to the primary analysis was 'controlled' to be this standard stepwise procedure (overall test of significance followed, conditionally on a positive result, by assessment of arm-to-arm contrasts). "Please see page 7.

4. The references to FODMAPs trials are not up to date.
Answer: The references were updated (page 17-21).

5. Some of the references are duplicates e.g. Hungin et al.
Answer: The duplicated references were removed.
6. I am not impressed that the correlations with QOL etc, add much to the paper.
Answer. We agree. However, the effect of IBS on health-related QOL is substantial;

therefore, we find IBS-specific QOL an important outcome measure in our study. We found no significant difference in QOL scores between the groups at 6 week.

Reviewer 4. *02531403*

Dear authors, I read with interest the manuscript written by Pedersen and coworkers, about the use of a low foodmap diet compared to LGG and natural western/danish diet in IBS. This is an excellent work, providing new and interesting insights for the management of IBS patients.

1. I would like to suggest to the authors to highlight the results of the analysis of covariance, in particular suggesting how the counselling (about smoking habits, the diet adherence, etc) may drive the attention of the clinicians towards the importance of both the diet and the lifestyle in the management of IBS.

Answer : We agree with the reviewer. The importance of patients´ counselling with respect not only to diet but also lifestyle (based on our results) is now discussed in Discussion section.

In our study former and current smoking was found to worsen the IBS-SSS symptoms. This is in agreement with previous results showing that smoking and other life style factors such as alcohol consumption and low levels of exercise have negative impact on IBS severity (LIU L et al. 2014. Biomed & Biotechnol).
Regarding the weight and BMI, all patients in our study had a normal BMI with no impact on IBS symptoms in all three groups. Interestingly overweight has previously been shown to be protective against the onset or worsening of IBS (Carter D et al. 2014. J Clin Gastroenterol), page 12

2. Please mention the role of the "classical" medication in this setting: the authors in the introduction define them as " a mild palliation", however how they could explain the positive correlation in their statistical analysis with the resolution of IBS SSS? I have no further remarks and I congratulate with the authors.

Answer: A little more than one third of patients in our study received conventional IBS medication such as laxatives, antispasmodic and antidiarrheal agents and antidepressants. Patients being on IBS medication and LFD in our study responded significantly better than patients on LFD without IBS medication.
The explanation of a better response on combined therapy in our study may reflect the complexity of IBS and the compliant patient (Saha L. 2014. WJG). Conventional IBS medication was shown to be more effective than placebo for treating IBS (Shah E et al. 2014. J Neurogastroenterol Motil) (ref. Ford A 2012) in short-term studies. A combination of these agents, switch between the preparations or combination with the dietary options might be more effective (Brandt J.There are limited data avaluable supporting the long-term safety and effectiveness of these agents. The role of IBS medication was discussed, page 12.

3 References and typesetting were corrected

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

Sincerely yours,

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