Author’s response to reviews

Title: Effect of indigestible carbohydrates in barley on glucose metabolism, appetite and voluntary food intake over 16 h in healthy adults

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Author's response to reviews: see over
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Some general comments regarding the revised manuscript:

After careful consideration regarding the reviewers comments, some changes have been made to the manuscript. Detailed comments to specific requests and questions are given in the following document. However, we would like to emphasize that in order to fulfill the requests concerning a better calibrated discussion towards the results, the discussion has been changed, mostly regarding disposition but also removal/addition of sections in the discussion.

Sections marked in grey indicates major novel/changes information.

In general, tables (4-6) presenting metabolic variables have been adjusted both concerning layout and content in order to facilitate the understanding of the results. Also, additional graphs demonstrating post-prandial responses have been added.

We hope that you will find the manuscript importantly improved.

Please find below our responses to the reviewers comments.

Reviewer Marion Priebe (MP):

Title: MCR
Perceived satiety: no difference in satiety was found.

We have changed the title accordingly.

Objective: MER
The objective of the work was to evaluate the potential prebiotic effect. As far as I know are dietary fiber in barley kernels not yet defined as prebiotics as they not yet have been shown to selective stimulation of the growth and/or activity(ies) of one or a limited number of intestinal bacteria beneficially associated with health and well-being - according to the definition of prebiotics (Roberfroid M, Prebiotic effects: metabolic and health benefits, 2011). Therefore the authors should not address the effect they are investigating as prebiotic effect.

The extremely confined definition of prebiotics as commented by MP is correct, and dietary fibre in barley is not included. However prebiotic potential of the barley substrates investigated have been suggested based on increase in intestinal microbiota in rats and in an in vitro model (Snart, Bibiloni et al. 2006; Maccaferri, Klinder et al. 2012). We have hence omitted mentioning of barley indigestible carbohydrates as prebiotic to be obedient with the current definition. However, we have kept a sentence in the conclusion in support of a potential prebiotic effect of barley DF and RS on basis of the current observations of beneficial metabolic effects linked to breath hydrogen in this work, and previously also to
plasma SCFA” *Taken together, the beneficial effects of BK are supportive for a prebiotic potential of intrinsic indigestible carbohydrates in barley based products.*”

“Specific attention was made to the possibility to increase .....incretin GLP-1.” GLP-1 was measured at the same time points as GIP, ghrelin and insulin. Therefore I do not understand in which way specific attention was given to GLP-1.

The specific comment regarding GLP-1 has been reformulated in the background (page 4).

**Materials and methods: MER**

**Study design:** What was the reason that the volunteers had to consume each evening meal twice but only at one occasion postprandial measurement were done? What was the method of randomization?

The study design included also a parallel series to secure previous findings with barley kernel evening meals in a situation of standardised breakfast meal. The results obtained came out similar to those already reported from our group (Granfeldt, Wu et al. 2006; Nilsson, Granfeldt et al. 2006; Nilsson, Ostman et al. 2008; Nilsson, Ostman et al. 2008; Nilsson, Ostman et al. 2008; Nilsson, Ostman et al. 2008) and others (Priebe, Wang et al. 2010). Thus, the data from this test series lacked originality but could be seen as an internal standard to the new experimental setting which is here reported for publication. The presently reported study add completely new knowledge, in that the “over-night” experimental setting has been extended to include not only breakfast, but also a lunch meal. Furthermore, as a novel approach, the influence of barley kernel evening meals as opposed to white wheat bread evening meals on metabolism over the course of 16 h was studied in an ad lib situation. The same test subjects participated both in the confirmatory series of experiment and the novel experimental design, and fasting values were therefore based on both visits. The randomization of the test and reference evening meals were performed in Minitab, and this is now mentioned more clearly in the revised manuscript (see Material and methods; Calculations and statistical methods).

**Calculation and statistical methods: MCR**

p. 10 “For calculations of fasting values.....two values for each subject were obtained, separated by at least 1 week”: I do not understand how fasting values can be obtained at different mornings as they should reflect the impact of the previous evening meal and are used to calculate the incremental AUC of the parameters of interest. Only fasting values should be used that are measured on the specific experimental day.

As described above, two fasting values were obtained for each test subject. However when calculating incremental AUC for different metabolic parameters such as glucose and insulin we have only used the fasting value of that specific occasion. We have clarified this in the text “Calculations and statistical methods” on page 9.

**Results: DR**

I do not find it necessary and also a bit confusing to indicate a “s” or a “p for measurements in serum or in plasma, while using f- for fasting. Mentioning in the method section whether plasma or serum is used seems enough to me.
The letters indicating plasma (p) or serum (s) is removed from the text and tables, but mentioned in the method section, as suggested by MP. The abbreviation indicating fasting (f) values are kept as before since it is of great importance to the reader to be reminded when fasting values are discussed, opposed to post-prandial values.

**Breath H2: DR**

Treatment and time x treatment effects are given in the graph, not necessary in the text.

Results for treatment and time x treatment are presented within the graphs of the revised manuscript.

**Blood glucose and insulin: MCR**

- The iAUC was calculated for the postprandial periods after breakfast and lunch but only an ipeak calculated for the postprandial period after breakfast. Why not for the period after lunch?

We agree that also ipeaks after lunch should be presented, and the time intervals of glucose and insulin should be better calibrated. Table 5 is adjusted accordingly.

- In the text it is reported that the iAUC is reduced during the entire experimental day after BK, however this is due to a significantly reduced iAUC after breakfast and not after lunch. This should also be mentioned.

We have added information regarding iAUC after lunch for both glucose and insulin (table 5). It should be noted that in addition to a significant difference in the iAUC after breakfast, there was additionally a statistically significant difference when investigating the whole experimental day, hence iAUC 0-330 min (glucose) with a decrease in iAUC after BK as compared to after WWB (main effects without interactions). When investigating the glucose iAUC after lunch (210-330 min) in table 5 and figure 2, the results also indicate a 15% decrease for BK compared to WWB, although not statistically significant. In the context of overnight studies, the time frame 0-330 min is not previously investigated, and therefore the reported effects on glucose and other metabolic variables are novel.

- Nothing is reported in the text about the postprandial insulin concentrations.

We have added a comment regarding the postprandial insulin concentrations to the text, as well as updated Table 5, and Fig 2 to include more details about the insulin response.

**Incretin hormones and ghrelin: MCR**

- GIP results are reported of the postprandial period 60 – 120 min. Is this iAUC?

The results of the other parameters are reported as iAUC from 0 – 120 min. Why is this reported differently?

The result for GIP 60-120 min is reported as AUC and this is now revised in the manuscript. The reason for reporting this specific timeframe is that we previously observed effects of GIP in this specific time frame (Nilsson, Ostman et al. 2008). Data for GIP are also adjusted to
match the timeframes previously reported for metabolic parameters (Table 6). The findings for GIP are also commented in the discussion, page 14.

- Ghrelin response is not significantly different but data should be provided.

Data for ghrelin is provided in the revised manuscript (Table 6, Fig 4).

**FFA, IL6, adiponectin: MCR**

Postprandial data of IL 6 and FFA are missing.

The postprandial response of IL-6 is illustrated in Figure 5. FFA was measured at time 0 and 210. The fasting values are presented in Table 3 and the results for time 210 min is now included in the Result section, page 11. Additionally, the results for FFA is presented in a section of its own rather than compiled together with inflammatory variables (IL-6 and adiponectin) as in the first manuscript.

**Subjective appetite ratings: MCR**

- According to the Method sections VAS where used to assess hunger, satiety and desire to eat, however only data of hunger are reported in the text (satiety in the table). What are the results of desire to eat?

There were no significant results for desire to eat. We have added Figure 6 to show the postprandial responses of satiety, hunger and desire to eat. Additionally results for Desire to eat are inserted in table 4.

- In the table AUC 90- 120 min of hunger was given – what is the rational for that? I would be interested in the rating shortly before lunch.

In the original manuscript the response of hunger 90-210 min was provided, that is for the period before lunch. However, after revising table 4 with information regarding desire to eat, the time frames for reporting subjective appetite ratings has been changed. Responses of appetite are now presented in 0-120 min, 120-210 min, 210-330 min and 0-330 min to better reflect the experimental day. The timeframe 120-210 is of specific interest since the late postprandial period after breakfast possibly influences voluntary energy intake at the subsequent voluntary lunch meal.

**Discussion: MCR**

- What is novel about the experimental design?

To our knowledge the time frame investigated in the present study 10.5-16 h after intake of test products, covering both breakfast and lunch, has not been studied before and is therefore novel. The serving of ad libitum breakfast and lunch is also a novel experimental design and allow conclusions in a more realistic eating setting. No such studies have previously been reported.

- “In agreement with previous findings [23,28], BK as an evening meal....” Reference 28 was not an overnight- study with BK, it was a 2wk intervention with
a prebiotic (fructan).

The discussion (page 13) has been changed to better reflect the references cited.

- “as a novel finding, BK decreased iAUC (0 – 330 min)”: glucose iAUC 0 – 120 min was significantly decreased while iAUC 210 – 330 min was not. I don’t think it can be described as novel finding because decrease in glucose iAUC 0 – 120 min was seen in several studies before: e.g. their reference 26 and 35 and Granfeldt Y, Wu X, Bjorck I. Determination of glycaemic index; some methodological aspects related to the analysis of carbohydrate load and characteristics of the previous evening meal. Eur J Clin Nutr 2006;60: 104–12.


It is correct that the differences in glucose iAUC 0-120 min are highly significant, and that the iAUC 210-330 min is not significantly different. However, even though not statistically significant, the mean value for glucose iAUC 210-330 show a 15% decreases after BK as an evening meal as compared to WWB. This difference is of course contributing to the fact that glucose iAUC 0-330 min show a significant decrease (no interactions time*treatment) in glucose response of 34%. As MP points out, the time frame 0-120 min has been intensively investigated by many research groups before, but in this study the time period is prolonged and therefore we consider this result as a novel finding. Also it should be noticed that unlike the design in the publications referred to above, the present study related to ad lib intake situation.

- “GLP-1 …has been suggested to account for up to 70 % of meal induced insulin release”, this is not right. 70 % of meal induced insulin release is the incretin effect thus a combined effect of GLP-1 and GIP.

Message taken and revision made accordingly (page 15).

- “Interestingly, studies in mice….increased levels of adiponectin improves clearance of serum FFA…” I do not see the relevance of this information for the discussion of this study: 1) f-adiponectin is the same while f-FFA are decreased, 2) no information is given about the postprandial FFA.

We agree, and the sentence is removed from the manuscript.

- The authors address the results of several 2 - 14 weeks intervention studies with oligofructans on gut microbiota and L-cell differentiation in the gut, contributing to higher GLP-1 production. Does this mean that the authors suggest that the same mechanisms exerting these long term effect are responsible for the effects seen 10,5 h after a single dose of barley? is there evidence for this acute
effect on L-cell differentiation?

The present human study has not examined L-cell differentiation as in the mice intervention. However, we find it interesting that semi-long term intervention studies in rats provided with prebiotic fibres have shown effects on metabolic parameters which coincide with those reported here following an over-night intervention and 16 h study period in healthy humans. The results of our study provide enough information to discuss possible explanations to the effects seen, i.e. relations between colonic events and host metabolism.

- One of the main objectives was to assess appetite and appetite regulatory hormones it is relevant also to discuss the discrepancy between the results of ghrelin (no difference) and the findings of the VAS as well as energy intake.

A discussion regarding ghrelin has been added to the manuscript (page 15). It should be acknowledged that several hormones are possibly involved in appetite regulation apart from ghrelin.

Reviewer's report
Title: Indigestible carbohydrates in barley as modulator of glucose metabolism, perceived satiety and voluntary food intake over 16 h in healthy adults - implicating colonic fermentation and stimulation of glucagon-like peptide-1
Version: 1 Date: 4 December 2012
Reviewer: gabriele riccardi (GR)

Reviewer's report:
Major Compulsory Revisions
1) This is an interesting paper on the impact of the evening consumption of barley, as compared with white bread, (both were utilized in portions containing 50 g available carbohydrates) on blood glucose levels, satiety, food intake and hormonal profiles evaluated in the following day. The major problem with this study is the interpretation of the findings which should be better calibrated on the results obtained.

We have acknowledged the comment and have changed the discussion section accordingly. For example, the discussion regarding GLP-1 has become more limited and the disposition has been adjusted.

2) The title attributes to GLP1 the role of major player in explaining the beneficial metabolic impact of barley; this is largely speculative and, therefore, it should not be proposed as an outcome of the study both in the title and in the conclusions.

In accordance to this comment, the title and the conclusions has been changed.

3) The test meal is based simply on the test or the control food rather than on a composite meal. This is a limitation of the study since the authors aimed at performing the metabolic evaluation during a “real life eating situation”. In the same line, it is unrealistic that they used a carbohydrate intake of only 50 g and
that the meal was taken rather late, at 9,30 pm.

The design of the study aims to develop the traditional approach to similar meal-studies. The “real life eating situation” is considered in the voluntary energy intake procedure at breakfast and lunch, preceding an evening intake of reference or control food. In order to investigate the impact of specific indigestible carbohydrates on the second and third meal, the test meals (reference and control food) are based solely on the amount of available carbohydrates (50 g available carbohydrates), as recommended for traditional glycemic index measurements (Brouns, Björck et al. 2005).

4) In the study design section it is stated that each evening meal was consumed twice; it is not clear what was the reason of this choice and how the results of the two tests were handled.

See response in the response to Marion Priebe, page 2 in this document.

5) In the paragraph on blood glucose and serum insulin there is no mention of the insulin patterns on the day after the two test meals; according to table 6, they do not show any significant difference. It should also be mentioned that no significant difference in postprandial plasma glucose values was present after lunch; this is even more remarkable considering that the energy and, possibly, the amount of carbohydrates was lower for the lunch taken on the day after the BK meal. Possible reasons for the inconsistency of the results after breakfast and after lunch should be considered in the Discussion (is it due to the time elapsed after the test meal?). It might be appropriate to evaluate insulin sensitivity after breakfast and after lunch using indirect methods based on insulin and glucose after a carbohydrate challenge.

Regarding the glucose response after lunch, there was a decrease by 15% (ns) contributing to the significant effect during the entire experimental day (0-330 min).

The lunch was more fat-dense in comparison to the breakfast, which may suppress the glucose responses – hence evening out differences. It is true that the subject consumed less for lunch after BK, which may have contributed to a lower glucose response. Nevertheless, in this “real-life” situation, when the subjects had an ad lib food intake, the fact that the glucose increments were lower after BK evening meal remain, regardless if the decrease depended on effects of colonic fermentation or a lowered intake of carbohydrates at lunch, or a mix of both. In any case, the differences responsible for the effects on lunch glucose increments must be related to the evening meal. We have extended the discussion regarding the results of lunch glycaemia on page 13). Information about insulin responses has been added (Fig 2 and table 5). No difference in HOMA-IR was detected (not presented in the manuscript).

6) The ghrelin values are missing from the text and from the table; if they do not differ between the two test meals, this should be commented in the Discussion: usually ghrelin is a good objective marker of the subjective feeling of appetite while GLP1 is more relevant for satiety.
Results and comments regarding the ghrelin response have been added (Table 6, Figure 4, discussion page 15).

7) FFA plasma concentrations were measured only before the meals: why? In any case, it might be interesting to know whether any significant difference in plasma FFA was present between the two test meals before lunch.

Previous studies (Nilsson, Granfeldt et al. 2006; Priebe, Wang et al. 2010) have not shown any post-prandial differences in FFA. However, findings in the fasting state have been reported (Nilsson, Granfeldt et al. 2006; Nilsson, Ostman et al. 2008; Nilsson, Ostman et al. 2008). The number of measurements in the present study was rather comprehensive and due to ethical limitations in sample volume, post-prandial measurements of FFA were not performed. There were no significant results in the response of FFA at time 210. Results regarding FFA at the time before lunch (210 min) is now included in the result section (page 11).

8) In the Discussion gut microbiota activity is often mentioned as a possible explanation for the study finding; as a matter of fact no evaluation of bacterial activity is attempted in this study. Breath H2 is a marker of colonic carbohydrate fermentation rather than of bacterial activity.

In the Background and Material and method, section Analysis of physiological variables, we clearly states that the measurement of breath H2 is used as an indicator of colonic fermentation, as stated by GR above. In the discussion, any conclusion regarding the microbial activity is always related to the measurement of breath H2. We have changed the wording in the discussion from gut microbial/colonic activity to gut microbial/colonic fermentation to better reflect the actual measurement. Nilsson et al. has previously investigated the impact of barley kernels on markers of fermentation and found that breath H2 and circulating levels of SCFA were significantly increased in the morning after barley kernel evening meals as opposed to white bread evening meals (Nilsson, Östman et al. 2010). These results strengthen the discussion of gut microbiota activity/fermentation as one possible explanation for the present study findings.

9) The role of GLP1 in explaining the study findings is overemphasized; GLP1 is often associated with satiety more than with hunger. In this study while GLP1 levels clearly differ between the two test meals for the entire duration of the observation, no difference in satiety is recorded and food intake is different for BK as compared with WB at lunch but not at breakfast. The importance of GLP1 in relation to insulin sensitivity is documented for pharmacological levels of this hormone but not for physiological changes, as in this case. Other parameters able to influence appetite and insulin sensitivity were not measured throughout the day (i.e. FFA, Short Chain Fatty Acids).

In the present study results for hunger reaches statistical significance. However, the late post-prandial response (AUC 120-210 min) of satiety reach the same numerical percentage change as for hunger (Table 4). The explanation to this apparent inconsistency is most likely that the study might have been under-powered with respect to VAS ratings. The result in the present
study provides distinct information that ingestion of specific indigestible carbohydrates has effects on metabolic responses in this relatively short time frame, and has profound effects on the plasma GLP-1 levels at fasting and over-the course of the study day, that is up to 16 h after the test barley evening meal. This in combination with evident responses of e.g. breath H2, indicates the role of the colonic fermentation in modulating the hosts metabolic responses to food. We think that the findings in the present, and previous works, gives enough basis to discuss GLP-1 to be possible involved in generating metabolic benefits. Previous publications from A. Nilsson have shown that providing barley kernels as an evening meal significantly increases levels of circulating SCFA, specifically butyrate, in plasma the next morning (Nilsson, Östman et al. 2010). Measurement of SCFA as an indicator of gut microbial activity is a relevant and interesting approach, which will be considered in future studies.

10) Conclusions are not entirely based on data; the final paragraph of the Abstract gives a more appropriate interpretation of the study outcomes.

We agree on the comment and the conclusions have been modified accordingly.

**Discretionary Revisions**

11) It is not clear why finger-prick capillary blood samples were utilized for blood glucose measurements while venous plasma was used for all other biochemical variables.

It has previously been stated, both elsewhere and in our group (Wolever, Jenkins et al. 1991; Granfeldt, Hagander et al. 1995) that the use of capillary blood versus venous blood for measurement of blood glucose levels allows for detection of smaller differences. Capillary blood glucose determination is the recommended praxis in GI calculations (Brouns, Björck et al. 2005).

12) In the Results section I find more logical to have the paragraph on subjective appetite ratings soon before that on voluntary energy intake.

The comment has been considered, but for the sake of comprehension, the arrangement of the results is kept as in the original manuscript.

13) Fig 1 does not contribute any information not already in the text; therefore, it can be deleted.

Figure 1 has been deleted and the information is provided in Material and Method, section Study design (page 6).

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

no conflict of interests
References:


