Author's response to reviews

Title: A bilberry drink with fermented oatmeal decreases postprandial insulin demand in young healthy adults

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Author's response to reviews: see over
Cover letter

Dear Editor,

We are truly grateful for the rigorous and serious evaluation of our paper. We have now considered all the points raised by the referees as stated below in blue.

Kind regards

Reviewer 1

Major compulsory revisions

1. Is the study underpowered? Analyses were performed on a relatively small number of volunteers and it may become significantly different with a larger sample size.

   We agree that the result might have been significantly different with more participants in the study. However the costume in studies measuring glycaemic index is to have ten participants, this is also recommended by the ILSI Expert group on glycaemic index methodology (Brouns et al Glycaemic index methodology, Nutrition Research Reviews 2005 (18) 145-171)

2. How is the sensitivity of the insulin measurement?

   The sensitivity is good (0.399 µU/ml); we have many years of experience from analyzing plasma insulin in small capillary samples with this enzyme-linked immunoassay kit (Mercodia Insulin Elisa; Mercodia AB, Uppsala, Sweden). The yellow part has been added in the revised manuscript.

3. Statistically significant is statistically significant; once one has set one´s rule one should live with it (lines 187-189, 200-202, 227-234, Table 3-4)

   We agree that the p-value has to be less than 0.05 to make the results statistically different. However we don´t think that has to exclude us to point out tendencies as we do on the indicated lines and in table 3 and 4.

4. Is the relationship between the insulin and glucose response sufficient to characterize the insulin demand? Is this method for calculating the insulin demand evaluated?

   “Insulin demand” is used to discuss the required amount of insulin necessary to achieve a certain- or in this case a similar degree of blood glucose control. As far as we know there is no generally accepted definition of insulin demand

5. How was GI and II calculated? More detail required.
In the revised manuscript we have added a paragraph (marked in yellow) with details regarding how GI and II were calculated “The incremental areas under the curves (AUCs) were determined for blood glucose and serum insulin (GraphPad Prism version 4.03; GraphPad Software, San Diego, CA, USA). GI and II were calculated from the area under the glucose/insulin response (0-120 min) after consumption of 50 g of carbohydrates from a test food divided by the area under curve after consumption of 50 g of carbohydrates from white wheat bread (reference) and with each subject being their own reference. All areas below the baseline were excluded from the calculations.”

6. How is GI calculated according to digestible carbohydrates? More details required. Is this method evaluated?

See answer point 5. Yes the method is evaluated (Brouns et al Glycaemic index methodology, Nutrition Research Reviews 2005 (18) 145-171)

7. Is there any difference in fat, fiber and protein content between the test and reference meal? Please clarify how the differences may affect the insulin-lowering effect.

The most pronounced difference is in dietary fiber content between BFOMB and BBFOMB (0.9 and 4.4 g/meal). There are only small differences in fat, fiber and protein content between the test and reference meals, not likely to affect the insulin-lowering effect.

8. Is there any difference in fiber content between BFOMB and BBFOMB? Please clarify how the difference in content may affect the insulin-lowering effect.

See above

9. The authors suggest that the postprandial insulin changes observed are due to changes in increased uptake of glucose into the peripheral cells. In the discussion the authors state that “no differences in glucose- or insulin responses demonstrating that oat beta-glucan reduces postprandial glucose and insulinaemic responses in type 2 diabetics and in non-diabetic subjects. A dose-response relation has also been observed between the amount of oat beta-glucan and the decrease in glucose and insulin levels in healthy subjects and in type 2 diabetics. Such work has not even been mentioned in the discussion.

We are aware of that beta-glucans from oats (in sufficient amounts) may reduce postprandial glucose and insulin responses (Granfeldt, Nyberg and Björck (2007) Muesli with oat beta-glucans lowers glucose and insulin responses after a bread meal in healthy subjects Eu J Clin Nutr April 1-8). However, in the current study we are referring to one of our previous studies showing that naturally occurring viscous dietary fiber in oats don’t affect postprandial glucose- or insulin responses (Granfeldt YE, Hagander B, Björck IM: Metabolic responses to starch in oat and wheat products. On the importance of food structure, incomplete gelatinization or presence of viscous dietary fibre. Eur J...
Clin Nutr 1995, 49:189-199. There are no beta-glucans added to the oatmeal used in the tested drinks.

There are sections in the “introduction” (lines 59-65) and “Discussion (lines 321-316) that have not been referred sufficiently. This needs to be rectified.

The sentence with sugar consumption in Sweden is excluded in the revised manuscript (lines 59-65). A reference has been added on line 317 “Liljeberg & Björck (1998) Delayed gastric emptying rate may explain improved glycaemia in healthy subjects to starchy meal with added vinegar. Eu J Clin Nutr 52, 368-371”

10. The discussion should first address the main finding, explain this, and relate it to the hypothesis. The present version is not completely focused and addresses different results that are confusing (lines 229-234 and 245-253).

We don’t agree. The discussion starts with the postprandial glucose and insulin response which also was the purpose of the study - “The purpose of the present study in healthy subjects was to determine the glycaemic and insulinemic responses of fermented oatmeal drinks added with bilberry and rosehip”.

Discretionary reviews

1. Use only standard abbreviations; use of nonstandard abbreviations can be confusing. The abbreviations C2C12 and AMPK are not explained (line 307 and 309). C2C12 is explained in the revised manuscript AMPK is explained (line 264).

2. When beginning a sentence with number, spell out the number (line 134). We prefer to keep the number (2).

3. The small numbers, such as whole numbers smaller than ten, should be spelled out (line 90, 134). We prefer to use number (5 %), instead of spelling the number (five %).

Reviewer 2

Major compulsory revisions

1. In these studies, two references were used. White wheat bread was used in both studies for determination of GI and II. When compared to the reference bread, the fermented oatmeal drinks – both with or without bilberries – seems to show more favorable insulin responses. These results are extensively discussed and are also presented in the abstract. In the second study, a reference drink without berries was also used. There is a significant difference between the reference bread and the reference drink, but no statistically significant differences between the bilberry drinks and the reference drink. The authors should discuss these results
more thoroughly and present conclusions on the possible contribution of bilberries on the reduced insulin demand (also in the abstract)

We agree, and in the revised manuscript a sentence has been added in the abstract (result). “There were no significant differences between the bilberry drinks and the control drink (FOMD)”. Even though there are no significant differences between the bilberry drinks and the reference drink, the differences between the insulin responses after white bread and the bilberry drinks are more pronounced (II= 63 and 49) than the difference after white wheat bread and the reference drink without bilberries (II=76). That is why we conclude: “The mechanism for the lowered acute insulin demand is still unclear, but may be related to some bio-active component present in the bilberries, or to some extent to the fermented oat meal base.”

The possible effect of the fermentation process per se is discussed in the discussion line 291 – 297 (fermentation of oats). In the revised manuscript one new reference has been added, also showing a pronounced insulin decrease with a fermented oat product (“Alminger M, Eklund-Jonsson C: Whole-grain cereal products based on a high-fibre barley or oat genotype lower post-prandial glucose and insulin responses in healthy humans. *European Journal of Nutrition* 2008, 47:294-300”). Fermentation of bilberries is discussed on lines 297– 313.

2. The glucose and insulin responses of the bilberry and reference drinks seem to significantly differ only in early postprandial phase (the first 30 minutes), when sucrose and starch are digested and glucose absorbed in the intestine. However, this is totally ignored in the discussion of the results. Instead, the authors discuss that the mechanisms might include increased uptake of glucose into pheripheral cells (muscle cells, adipocytes) or improved insulin sensitivity. These mechanisms could influence glycaemia and insulinemia during the later postprandial phase, after absorption of glucose. The discussion of the possible mechanisms involved should be reconsidered.

It is true that at individual time points, significant differences were only obtained after 30 min. However, at AUC for the BBFOMD product was significantly lower that after the reference. In light of this we do feel that the mentioning of effects on peripheral cells is justified. See also the comments from reviewer 3 point 9

3. Statistical analysis: Statistical significance of fasting values, AUCs, GIs and IIs was analyzed with ANOVA and Tukey’s test (Table 3 and 4). Which test was used for analyzing the time x treatment interactions of glucose and insulin responses presented in Figures 1-4? Add this information also in figure legends.
The same statistical analysis (ANOVA and Tukey’s test) were used this is clarified and inserted in figure legends in the revised manuscript

4. The authors could comment on the reasons for the lack statistical significance especially in the cases where differences between the groups seem obvious, e.g. II 76 for FOMD and 49 for BBFOMD.

We agree that it seems to be a significant difference between FOMD and BBFOMD, however the p-value is 0.1011 and thus > 0.05, the limit for significance.

5. The figures are unsatisfactory, they should be redrawn. The timepoints given on the x axis could be the ones used in the experiments (0, 15, 30 etc). It is very easy to do these changes in Graph Pad Prism.

We don’t agree as long as the blood samples not were taken at regular intervals (15, 30, 45, 70, 95 and 120 min for glucose, and at 15, 30, 45, 95 and 120 min for insulin) it is not suitable for the x axis.

Minor Essential Revisions

6. Abstract, Conclusions: For Vaccinium myrtillus, the terms “blueberry” and “bilberry” are used. Please use consistently “bilberry”

We agree and this has been changed, however we prefer to include blueberry once in abstract to avoid obscurity and explain the similarities and differences between blueberries and bilberries. Further, the word “blueberries” is used if we refer to a study where blueberries and not bilberries were used.

7. Methods, third paragraph: The test meals were consumed within 12-14 min. After each meal, water, tea or coffee was served. Were they served before the 15 min blood sampling? Did you check that tea or coffee would not interfere with the glycaemic responses? Tea and coffee contain polyphenols which may influence digestion and absorption of carbohydrates and thereby postprandial glycaemia. Also caffeine is known to have an effect on the glycaemic response.

Yes, tea, coffee or water was served after each meal, before the first blood sampling at 15 min. The test subjects were allowed to choose between these drinks. However, they maintained with the same drink through-out the study, and the results base on differences between products.
8. Discussion, eight paragraph: There are many papers available on the content of anthocyanins and other polyphenols in the Scandinavian bilberries (for the replacement of reference 38). Reference 39 includes only phenolic acids, not all polyphenols common in berries.

Former reference 38 has been exchanged for “Prior RL et al: Antioxidant Capacity As Influenced by Total Phenolic and Anthocyanin Content, Maturity, and Variety of Vaccinium Species. J Agric Food Chem 1998, 46:2686 – 2693” and has phenolic acids been inserted instead of polyphenols

Minor issues not for publication:

Title page: Check the superscript numbers (1-5 are given after title, but only 3 and 4 are used in the addresses)

Done

- In several parts of the text, the phrase “respectively” is used incorrectly. Corrected
- All tables and the text: Don not use comma as a decimal point. Done
- Tables 3 and 4, footnote: Turkey´s test should be Tukey´s test Done
- Table 3, footnote: Should “comparing reference bread and fermented drink with RFOMD” be “comparing reference bread and BFOMD with RFOMD”? Corrected to “comparing reference bread with RFOMD”
- References:
  Check the spelling of the author names (e.g. ref 12, 24, 27, 28) Done
  Check the spelling of the article titles or the bibliographic information (e.g. ref 5, 9, 35) Done
  Use the abbreviated journal names (e.g. ref. 8,20,24,26,37) Done

Discretionary Revisions

9. Methods, third and fifth paragraphs (Subjects): These paragraphs could be combined to avoid repetitions. No, there are two studies with different subjects.

10. Tables 1 and 2 could be combined. Done

11. Figure legends: Because the symbols of the meals are presented in the figures, it is not necessary to repeat them in the legends. Done
Reviewer 3

**Major compulsory revisions**

**Minor essential revisions**

1. **Title, superscripts 1-5? I only find 2 authors on the page**
   
   Corrected

2. **Abstract, the use of the term blueberries, bilberries, European blueberries could be more consistent in the abstract, although some of the different species of the Vaccinium genus are described in the background, as the abstract is written now it can be a bit confusing and difficult to understand e.g.: a drink enriched with bilberries (10%) and a drink enriched with blueberries (47%) – are different berries used in different series? Suggestion: in the second sentence….. following dietary supplementation with extracts from European blueberries, also called bilberries (Vaccinium myrtillus)…..and then use the term bilberries in the rest of the abstract.**

   The revised manuscript are changed according to the suggestion, see also reviewer 2 point 6

3. **Background, lines 58-70. From Sugars are important components of the diet etc. The purpose of referring to the current dietary recommendations is not clear to me; do the authors consider it a risk of including 100 g of fruit juice with the recommended intakes of at least 500 g of F&V/day? If the purpose is to link the background description to the design of the study I think this need to be clarified or expressed a bit different?**

   We agree, the three following sentences are excluded in the revised manuscript;

   - Sugars are important components of the diet, providing about 10% of total energy in Sweden.

   - Current dietary recommendations emphasize generous amounts of fruits and vegetables. -- The daily recommendation in Sweden is at least 500 g of fruits and vegetables per day, including 100 g fruit juice.

4. **Background paragraph 3 (lines 87-92): a suggestion to write this more “concisely”: The present study was performed to determine the glycaemic and insulinemic responses in healthy humans after single meal intakes of fermented oat meal drinks containing different amounts of bilberries (0,10 or 47%) or rosehip (10%). (Perhaps it is not even necessary to include the % since it is described in the methods.**
We agree, in the revised manuscript the suggested sentence is used.

5. Methods, Paragraph 2 Series 1 and Paragraph 4, Series 2, No details or information regarding the processing or the “state” of the berries are included. As discussed later in the manuscript, anthocyanins in bilberries have been suggested to have an impact on postprandial responses although the metabolic pathways for this are not clear. Based on the current literature it is widely accepted that berry phenolics/anthocyanins are poorly bioavailable but it is possible that food processing can improve (or limit) the uptake in the gastrointestinal tract, even if they are not absorbed in the small intestine they can be metabolized in different ways in the colon, possibly depending on the “stat” and the food matrix. Thus it would be interesting to know whether the bilberries and rosehip were fresh, frozen dried or had been submitted to some other processing. (more than homogenized as described in series 2).

It has been clarified that the bilberries used were frozen and thawed before homogenized.

6. Methods, Subjects, from this paragraph and further on in the text (methods, results, discussion) the authors alternate between min or minutes, for consistency the same should be used (suggest min).

We agree, in the revised manuscript min is used as suggested

7. Methods, Sampling and analysis. Samples for analysis of glucose were taken at 15, 30, 45, 70, 95 and 120 min, although this may not have a major effect on the results, it would be interesting to know why the “standard method” for taking blood samples for determining glycaemic responses was not used in this study i.e. with samples usually taken at 15, 30, 45, 60, 90 and 120 min?

This was done only for logistical reasons as we had several subjects involved, in this way we spread the time points for sampling. We don’t think this has to be mentioned in the manuscript.

8. Methods, Sampling and analysis and Results, Series 1 paragraph 3: series 2, paragraph 5. The insulin concentration was not determined at 70 min, the postprandial insulin concentrations clearly peaked at 45 min (Figs 2&4) but 50 min seems to be quite long period between the 45 and 95 min time points for assessment of the shape of the insulin curve?

We agree, however we didn’t realize the interesting insulin results in advance

9. Discussion, the discussion is very interesting and especially the hypothesis that bilberries can cause an increased uptake of glucose into peripheral cells and the reference of potential activation of AMPK in type 2 diabetic mice from bilberry extracts. However in paragraph 5 of
the discussion, the authors suggest that the inconsistency between glycaemic and insulinenic responses was unexpected and only previous reported for certain rye products and for cinnamon added to rice pudding. According to my present knowledge, the inconsistency between GI and II is in line with other previous studies (Flint et al. Br J Nutr. 2004; Mäkeläinen et al. Eu J Clin Nutr. 2007; Alminger & Eklund-Jonsson, Eur. J. Nutr. 2008)

We agree that Flint et al and Mäkeläinen et al also reports an inconsistency between glucose and insulin, but with unexpected high insulin, responses. However, Alminger and Eklund-Jonsson reports a lower insulin response in parallel to a higher glucose response for fermented whole-grain oat, this article has been referred to in the revised manuscript.

10. Table 3&4, Misspelling of Tukey´s test

Corrected