Author's response to reviews

Title: Soy foods have low glycemic and insulin response indices in normal weight subjects

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Author's response to reviews: see over
Dear Sirs,

Thank you for your thorough review of and comments and suggestions regarding our manuscript “Soy Foods Have Low Glycemic and Insulin Response Indices in Normal Weight Subjects” (Manuscript #1504602090970674). Our point-by-point responses are listed below each review point and the appropriate changes have been made in the manuscript.

We hope you will find these responses and changes satisfactory and hope you will find the revised version of the manuscript acceptable for publication in the Nutrition Journal.

Best Regards,

Robert M. Blair, Ph.D.
Research Manager,
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Major Compulsory Revisions

The title of the manuscript ("Soy foods have low glycemic and insulin response indices") suggests that the results obtained in the study can be attributed to the soy content of the food tested. However, in table 1 and 2 no information about the soy content of the food is provided.

Information on the soy content of the food has been added. Please see our response to Item #1 below. Since all the protein in the foods tested was soy protein, with the exception of the soy spaghetti, we feel that the title of the paper remains appropriate.

1. The amount of soy used to manufacture the food tested in this study should be provided (Method section). Otherwise it is not possible to judge if or to what extent the results obtained can be attributed to the soy component of the food. According to the Instructions for Nutrition Journal authors "Reports of clinical research should, where appropriate, include a summary of a search of the literature to indicate why this study was necessary and what it aimed to contribute to the field". This should be done avoiding excessive referencing.

All of the protein listed in Tables 1 and 2 is soy protein with the exception of the protein found in the soy spaghetti. For the soy spaghetti, 57% of the protein used is soy protein; the remaining protein comes from the semolina used to make spaghetti. The amount of
soy protein used to manufacture the food tested in this study has been added below each table.

A brief summary of our literature search has been included in the Methods section on page 5.

2. The use of 69 references with an original research report is pretty excessive. It is not necessary to quote most of the original research articles to provide a summary of the relevant literature. This can be done by referencing some of the recent reviews on the topic. The references used in the current manuscript should be reduced to a total of about 20 of the most relevant articles (see also comment no 7). In order to obtain reliable GI values of carbohydrate containing foods, it is necessary to use a standardized methodology for the GI measurement. The FAO/WHO expert consultation has, therefore, recommended a standard GI methodology in their report published in 1998, and recently this recommendation has been updated by Brouns et al (Nutr.Res.Rev. 18:145-171, 2005).

The number of references has been reduced to 53, which is more in alignment with the editorial policy stating “there should usually be no more than 50 references per article”. It is our opinion that while citing predominantly review articles can be useful and is often times appropriate; it does not give credit to the original researchers and their publications. Therefore, we would prefer to cite original papers over review papers where possible.

We have included the Brouns et al. paper as suggested in our reference list.

3. It is stated on page 5 (Methods) that the current study was conducted using internationally recognized methodology (referencing the FAO/WHO report), validated among others by a large multicenter study (referencing Wolever et al. Eur.J.Clin.Nutr. 57:475-482, 2003). In the FAO/WHO report it is recommended to use the incremental area under the curve (IAUC) for the calculation of the GI and the GI was calculated accordingly in the interlaboratory study by Wolever et al. However, on page 7 last line it is stated that the area under the curve (AUC) was used for GI calculation in present manuscript, which is not in accordance with the recognized methodology. Furthermore, the methodology validated by the interlaboratory study refers to an amount of 50 g available carbohydrates with the reference and test food. In the present study portions were served containing 10 g net carbohydrates (Exp. 1) and 25 g (Exp. 2). Thus, it is 1) not correct to use the term GI for the glycemic responses obtained in the present study and 2) not correct to state that the study was conducted using internationally recognized methodology. The term "glycemic index" used to describe the glycemic response measurement with this study can, therefore, not be used as long as the AUC calculations are not replaced by IAUC calculations. The GI methodology review by Brouns et al (Nutr.Res.Rev. 18:145-171, 2005) recommends to use portions
containing 50 g of available carbohydrates for GI measurements and states that for foods with low carbohydrate it is justified to lower this amount to 25 g. In Experiment 1 of present study a portion was used that contained only 10 g net carbohydrates. Since an amount of 10 g of available carbohydrates is neither recommended by the FAO/WHO report nor by the GI methodology review, the authors need to provide evidence that such a low carbohydrate amount is suitable to reliably measure the glycemic response/GI. If no evidence can be provided, the results of Experiment 1 cannot be reported under the term "glycemic index".

The area under the curve data used to calculate the glycemic index was in fact the incremental area under the curve. The misuse of the terms AUC and IAUC was an error on the part of the author and has been corrected throughout the manuscript.

The review by Brouns et al. (2005) indicates on page 154 that equal available carbohydrate portions should be used for GI testing purposes. In our study, smaller portions were required due to the very low available carbohydrate content of the test foods; however, equal available carbohydrate portions of the test and reference foods were utilized. The Brouns paper discusses the use of smaller test portions (page 155) and recommends that for low-moderate carbohydrate containing foods, the use of lower carbohydrate testing portions is justified in order to avoid unrealistic food amounts as long as an equal available carbohydrate portion of the reference food is also used. Brouns goes on to say that less than 25 grams of available carbohydrate may be considered for foods with very low available carbohydrate content. The only concern expressed was that testing of less than 10 grams of available carbohydrate might result in the lack of a significant change in blood glucose. Since we did not use less than 10 grams available carbohydrate and since we were able to detect changes in blood glucose, we believe that the data generated with 10-gram portions is valid.

4. On page 7 line 1 it is stated that the experimental method used in the current study has been previously described (referencing Chan et al Eur.J.Clin.Nutr. 55:1076-1083, 2001). However, since Chan et al used portions that contained 50 g available carbohydrates (in contrast to 10 g or 25 g used in current study), which represents an important part of the experimental method, it is not correct to state that the current study was done as described by Chan et al. According to the GI methodology review by Brouns et al, glucose analysis methods with a CV > 3% should not be used for scientific purpose. Accordingly, information about the glucose determination is crucial to judge its feasibility for the GI assessment.

A more appropriate reference where levels of available carbohydrate below 25 g were tested has been used in place of the Chan 2001 reference.

The glucose concentrations in each of the whole capillary blood samples was analyzed in duplicate using a glucose dehydrogenase/mutarotase enzymatic reaction using a
HemoCue beta-glucose photometric analyser. Duplicate readings were accepted if the two separate measurements for each time point were within 0.3 mmol/L of each other. If the readings were not within 0.3 mmol/L of each other, then an additional 2 blood sample readings were taken from the subject within approximately 40-60 seconds after the initial readings. The two or three similar (i.e. within 0.3 mmol/L) readings were then averaged together to obtain the blood glucose response for that time point. This information has been added to the methodology in the paper.

5. Information about the glucose analysis should be extended on page 7. The CV of the method used should be mentioned and information about the differences of the duplicate measurement should be given. When measuring many blood samples the glucose values of duplicate measurements sometimes diverge greatly (more than the CV of the assay). With how many duplicates did this happen? What was the procedure with these cases? Third measurement? The extensive discussion of the literature related to a particular topic represents a necessary part of a review article. However, in an original research article the discussion should focus on the interpretation of the results obtained by comparing them with relevant data from the literature. An extensive review of the literature has to be omitted.

Please see response to Item #4 above in regards to glucose methodology.

The literature review in the Discussion has been limited as suggested.

6. The discussion from page 11 last paragraph until and including page 14 first paragraph should be reduced to maximum one paragraph shortly discussing the pros and cons of the GI and GL concept. Since the article is concerned about the health effects of soy, both the potential positive and negative effects of soy consumption should be discussed. This is only very briefly done on page 14.

The Discussion from page 11 through the top of page 14 has been dramatically reduced as suggested and the corresponding references omitted.

7. The second paragraph on page 14 could be somewhat extended by discussing the potential positive but also potential negative health effects, such as the catabolic effect of soy protein on nitrogen balance (Bos et al J.Nutr. 133:1308-1315, 2003), which is usually omitted when discussing the health effects of soy.

The information on the health effects of soy has been expanded upon as suggested.

Minor Essential Revisions
8. On page 4 the second paragraph starts with the statement that high GI/GL diets have negative health effects. However, in the following two sentences it is stated that the risk for diseases might be increased. This inconsistency should be clarified.

_We have tried to clarify this first sentence by changing it to read “…a high GI diet may have adverse health consequences by increasing the risk for chronic disease”. We did not mean to imply any immediate “adverse health consequences”._

9. Page 5 1st paragraph: the abbreviation II for insulin index is used although it has not been previously defined (definition is on page 8).

_Thank you, this has been corrected._

10. Page 5 1st paragraph: the information about the market is missing (US market?)

_Corrected to read the “US and international markets”._

11. Page 5: the country name of the Ethics Committee is missing.

_The country (Australia) has been added._


_Corrected._

13. Same line on page 5: What denotes the age range mentioned (18-45 y)? Absolute age range or 95 % CI range? The age ranges of the subjects in the two experiments (19.9-25.7 y; 20.3-26.9 y) are not in agreement with the age range of 18-45 y. This should be clarified.

_The age range (18-45 y) in this sentence was removed. This age range was part of the inclusion criteria._


_This information has been added to the first paragraph under the section Study Subjects._

15. Page 6 1st paragraph: no indication is given about the BMI range. Absolute range?
Yes, the BMI range is the absolute range. This has been added to the paper.

16. On page 6 2nd paragraph net carbohydrates are defined as total carbohydrates-other carbohydrates-fibre. Since neither 'net carbohydrates' nor 'other carbohydrates' are commonly used terms, the authors should provide a definition of these terms.

"Net carbohydrates" are considered those carbohydrates that are available and have an impact on blood glucose. "Other carbohydrates" are those carbohydrates that do not have an impact on blood glucose. In this instance, we are talking about sugar alcohols, so the term “other carbohydrates” has been replaced by “sugar alcohols”.

17. Page 6 2nd paragraph: the country name of the company providing the glucose powder is missing.

The country name (Australia) has been added.

18. Page 6 2nd paragraph: "Weights and nutrient contents..." should be changed into "Mass and nutrient contents...", since weight denotes a force with the unit Newton. The measure with the unit kg is 'mass'.

Fixed. This has also been corrected in the titles of Tables 1 and 2.

19. Page 6 2nd paragraph: The composition of the test food in table 1 & 2 should be also given in nutrient per 100 g of product to allow for a general comparison among the different foods tested and with foods in general.

This information has been added to the tables as suggested.

20. Page 6 last paragraph: it is stated that reference and test foods were served with 250 g water. However, in table 1 and 2 the amounts of water range from 0 to 500 g. This inconsistency should be clarified.

The amount of water listed in Tables 1 & 2 is the amount required to prepare the food products (i.e. subjects did not eat the shake powder dry, but added water to prepare the shake). In addition to the water used to prepare the product, each subject consumed 250 grams of plain water with the test or reference food. A footnote at the bottom of each table has been added to clarify this point.

21. Page 7 2nd paragraph: the abbreviation after the company name should be GmbH with capital H at the end.

Corrected
22. Page 8 2nd paragraph: AUC was already defined in the 1st paragraph of page 8.

*Fixed.*

23. Page 9 last paragraph: How was the correlation between the insulin and glucose response curves calculated? No information about correlation analyses is given in the Statistical Analyses section and the curves on figure 1 do not look particularly 'correlated' to the curves on figure 2.

*No correlation analysis was done for this study and I cannot find any reference to correlations in the manuscript. We did indicate the glucose and insulin responses were proportional, but this was in reference to the curve profiles as described in the 3rd sentence of the last paragraph on page 9 in that both Zing bars had both higher glucose and insulin responses than the Daydream shake. We have re-worded the second sentence in hopes of clarifying this point.*

24. Page 9: The term 'Non-low-carbohydrate' looks pretty odd. It is suggested to change it into high carbohydrate or medium carbohydrate, according to what is more appropriate.

*Corrected.*

25. Page 30&31, Figures: Information about the significant differences between the foods should be provided with the figures.

*This information has been provided with Figure 5 and the legend for Figure 5.*

26. Page 32&33: The term weights should be changed into mass.

*Fixed.*

27. Page 34: Information should be given about the amount of carbohydrates used in the portion to assess the glycemic response. SEM is not defined in the footnote of the table. A reference for the GI & GL categories is missing.

*The amount of carbohydrates used in the portion to assess the glycemic response has been added to the table and SEM has been defined in the footnote.*

*These ranges are based on the Sydney University Glycemic Index Research Service (SUGiRS) ([www.glycemicindex.com](http://www.glycemicindex.com)), the group run by Dr. Jennie Brand-Miller that was contracted to measure the glycemic index of the test foods. The same ranges are used by the Glycemic Index Laboratories ([www.gilabs.com](http://www.gilabs.com)), which is run by Dr. Thomas Wolever. For citation purposes, we cited the SUGiRS website, because to our*
knowledge there is no scientific paper that has published classification of low, medium, and high.

28. Figure 2 & 3 & 4: "…Shakes" with no final s.

Corrected.