Title: Soy isoflavones increase preprandial peptide YY (PYY), but have no effect on ghrelin and body weight in healthy postmenopausal women

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Version: 2 Date: 3 August 2006

Author’s response to reviews: see over
Weickert MO et al.  
“Soy isoflavones increase preprandial peptide YY (PYY), but have no effect on ghrelin and body weight in healthy postmenopausal women”

We are grateful for the constructive comments of reviewer 1. Please find below a detailed response to the concerns of this reviewer.

**Major Compulsory Revisions**

*ad (1): Several grammatical errors throughout the manuscript.*

We have screened the complete manuscript for grammatical errors. The specific points suggested by the reviewer have been addressed as follows:

Page 2:  
“and probably also” has been replaced by “and”
“may show potentially present smaller differences” has been replaced by “might detect smaller differences”
“indicative for a...physiologically increased”. We rephrased this sentence.

Page 3:  
“and has been discussed to be of potential therapeutic use” has been replaced by “with potential therapeutic use”.

Page 7:  
“However, increased protein” We have rephrased the first two sentences of the discussion.

Page 8:  
“showed not any effect” has been replaced by “did not affect”
“physiologically increased”. We deleted “physiologically increased”.

Page 9:  
“in order to standardize for” We rephrased this sentence.

Page 11:  
“Normally distributed data” We rephrased this sentence and the following sentence.

*ad (2): Other recommendations:*  

**Page 5 and 11: Choice of statistical analysis:**

We used a linear mixed model to show changes of effects between groups, after adjustment for changes in BMI. This method was chosen because we intended to show changes in effects separately for the treatment groups. For the comparison of mean values (and with adjustment for covariates) we now also calculated ANCOVA, as suggested by the reviewer (please see below). The results are comparable to the results received by the linear mixed model method.
However, because ANCOVA compares the means, but does not allow to detect changes in effects, and because subjects cannot be included as random factors with this method, we would prefer to present only results from linear mixed model analysis in the final version of the manuscript.

Please see the essential results from ANCOVA analysis below. “Delta” gives the difference between week 8 and week 0:

- There was no effect of treatment on BMI (P = 0.333). Delta ghrelin (wk8 – wk0) was not dependent on treatment (p = 0.304), and there was an effect of baseline ghrelin (P = 0.038) and Delta BMI (p = 0.03). Delta glucose was dependent on Delta BMI (P = 0.033) and baseline (wk 0) glucose concentrations (P < 0.005). Delta insulin was only dependent on Delta BMI (P = 0.012). Delta PYY was dependent on the treatment (P = 0.009) and on baseline PYY concentrations (P < 0.005). There was no effect of Delta BMI on Delta PYY.

**Why was BMI used to adjust the results? Did BMI explain a significant amount of variance?**

Data were adjusted for changes in BMI, but not for BMI. We are grateful for this comment and corrected this sentence in the Methods section (page 11, Statistical Analyses). Adjustment for changes in BMI were necessary because of known effects of changes in BMI on concentrations of glucose, insulin, PYY, and ghrelin.

**Why certain fixed effects always in the model?**

There was a statistical meeting of the ISOHEART partners, and all project partners agreed to use the given fixed effects to standardise the procedures (Baseline values were included because higher baseline values might lead to more pronounced changes in some of the parameters. Treatment order was included to control for seasonal variations. Changes in BMI were included because body weight was correlated with all variables (PYY, ghrelin, glucose, insulin), and thus it can not be excluded that a change in BMI might cause a change in PYY and ghrelin concentrations).

**Which data were log transformed?**

Skewed data were log transformed. We included a sentence in the Methods section.
Page 6: *If equol production was a significant factor in ghrelin, was this used as an independent variable...*

Equol production status was included as an independent variable in the model. As suggested by the reviewer, correlations of ghrelin with BMI are weak. Therefore relevance of this finding is doubtful, and we do not report this correlation in the revised version of the manuscript.

*What was the power to detect ghrelin and PYY?*

The estimated power was 86 percent to detect a difference of 15 percent in ghrelin concentrations, with a sample size of 34 subjects, a SD of 40 pmol/L, and a significance level of 0.05. However, because the observed SD was higher, a potential difference in ghrelin concentration might not have been detected with the sample size of 34 subjects. The estimated power was 89 percent to detect a difference of 10 percent in PYY concentrations, with a sample size of 34 subjects, a SD of 10 pmol/L, and a significance level of 0.05.

Page 7: “*However, increased protein ... The additional protein in the cereal bars was 5.2 g/day*”

We agree that our statement needs to be clarified. Additional protein intake was very low, and identical in the treatment and placebo group. The only difference between the diets was the isoflavone enrichment of the cereal bars. The statement “However, increased protein…” referred to studies that investigated effects of soy food on body weight, and the increased protein intake by consuming soy food, rather than the increased isoflavone intake, might have caused effects on body weight. We now have clarified the respective sentences to avoid confusion. (page 7, first two sentences).

Page 8: “*One of the important findings ...*”

We are grateful for this comment and included the following sentence in the discussion: “In addition, high within and between subject covariations in preprandial ghrelin concentrations have been reported, which may lead to the detection of random effects rather than of true treatment effects”.
Page 9: “Please justify your selection of the quantities of isoflavones / protein in the cereal bars”

The daily intake of isoflavones in Asian populations consuming a traditional soy-rich diet is 15 – 50 mg/d. In Western populations, isoflavone intake has been reported to be as low as < 1 mg/d (de Kleijn J Nutr 2001). Therefore, soy isoflavone intake in the present study was at the upper limit of daily isoflavone intake in traditional Asian diets. We included this statement in the Methods section (page 10). As already stated (please refer to comment “Page 7,…increased protein intake”), protein doses of the cereal bars were identical between the treatment and the placebo groups. Low doses of protein in the cereal bars were appreciated, because higher protein contents might have obscured potential effects of isoflavones on satiety.

Minor Essential Revisions

Page 5 : “Please reference which technique was used to assess food intake”

Diet diaries were completed at baseline (t0) and after 4 weeks (t4) of each intervention arm. All food records included two week days and one weekend day. Nutrient intake was calculated based on the German Food and Nutrient Data Base Bundeslebensmittelschlüssel (BLS II.3). … Subjects kept daily records of cereal bar consumption and well-being in a study diary. Dietary compliance was further assessed by measurement of phytoestrogen concentrations in 24-hour urine. This was stated in the Methods section (page 10).

“Only use 2 significant digits for r-values.”

We now report r-values with two significant digits in the revised version of the manuscript (page5).

“Please detail the energy and macronutrient composition of the evening meal”

Subjects were provided with a choice of different recipes, which were all designed to provide a fat content of less than 10 g (fat contents were calculated in PRODI). We included a sentence in the Methods section (page 9).

Please explain the purpose of Figure 1

We have deleted this figure, because we agree that additional information is limited.
Page 10: Please report the within / between subject CV’s for ghrelin.

Please see below. Please note that these values are log transformed and given in ng/mL.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ghrelin wk0 (ng/ml)</th>
<th>Ghrelin wk8 (ng/ml)</th>
<th>Delta Ghrelin wk8-wk0</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Placebo</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>2.602154</td>
<td>2.569145</td>
<td>-0.020652</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>0.239459</td>
<td>0.2385793</td>
<td>0.0931305</td>
</tr>
<tr>
<td>Variance</td>
<td>0.057</td>
<td>0.057</td>
<td>0.009</td>
</tr>
<tr>
<td><strong>Isoflavone</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>2.557074</td>
<td>2.568291</td>
<td>0.011217</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>0.2551999</td>
<td>0.2490913</td>
<td>0.0712718</td>
</tr>
<tr>
<td>Variance</td>
<td>0.065</td>
<td>0.062</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>2.579278</td>
<td>2.568718</td>
<td>-0.004480</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>0.2467381</td>
<td>0.2420654</td>
<td>0.0836825</td>
</tr>
<tr>
<td>Variance</td>
<td>0.061</td>
<td>0.059</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Discretionary Revisions

Page 2: “Changes in …. I am not sure why this statistic is reported…”

We deleted this sentence in the abstract and in the Results section (page 6). We report r-values with two significant digits in the revised version of the manuscript (page 5).

- Please note that SDs in table 2 are now given as SEMs.