Author's response to reviews

Title: Postprandial energy metabolism and substrate oxidation in response to the inclusion of a sugar- or non-nutritive sweetened beverage with meals differing in protein content

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Author’s response to reviews:

We would like to thank the reviewers for their time and critical review of our manuscript. We believe that the comments and requested changes have provided a great service in helping us to improve the manuscript.

Reviewer reports:

Reviewer 1: Well written manuscript, and very well controlled human studies that add nicely to the scientific literature.

Specific comments:

page 5, line 77: The abbreviation NNSB was defined in the abstract but not again within the body of the manuscript. Perhaps define again here for the reader.

- This oversight has been corrected.

Was habitual physical activity considered and controlled in research design? Please describe accordingly. If participants were physical activity, was this a factor in the analysis of data?

- Physical activity was only controlled for the 2 to 3 days prior to each study visit. Participants were instructed to maintain their activities of daily living. We did not have the participants wear an activity monitor, so we do not have an objective measure of their usual activity. As such, usual activity was not included as a factor in the data analyses. However, physical activity was measured by questionnaire and importantly those self-report results did not differ between test visits and have been added to the manuscript (page 15, lines 287-390).
Habitual macronutrient composition has profound effects on fasting substrate oxidation and energy expenditure. Were research participants instructed to adhere to specific dietary patterns during the 1-8 week period between the testing periods. If so, please describe. If not, perhaps consider as a limitation in discussion.

- Participants were instructed to maintain their habitual diets. Data from 3-day food records showed nonsignificant differences in energy intake and macronutrient composition from visit 1 to visit 2. Energy intake only differed by 22.7 kcals, carbohydrates by 0.24%, fat by 0.18% and protein by 0.72%. We have added these data to the manuscript (page 15, lines 280-283). Thus, we do not believe that these minimal differences in the participants’ habitual dietary intake contributed to the differences observed in substrate oxidation and energy expenditure in response to the dietary intervention.

Was physical activity controlled during 1-8 period between test periods?

- Please refer to our response to your question about physical activity.

Were analyses performed to assess the relationship between habitual macronutrient composition and metabolic responses to test diets? Perhaps consider.

- We have performed an analysis to assess the relationship between habitual macronutrient composition and metabolic responses to test diets. This analysis has been added to the results (page 19, lines 363 – 370). We have also added a statement about this limitation (page 24, lines 490 – 494).

What standards were used to assure energy balance conditions during the testing periods? Please describe.

- The meals were not adjusted based on individual energy needs. This was an acute study and all meals were comprised of the same foods and provided 500 non-beverage kcals (page 9, table 2).

Reviewer 2: BMC NUTRITION - MANUSCRIPT REVIEW JANUARY 2017 - SL Casperson et al.

This manuscript describes a double-blind randomized crossover study in which 27 adults were each tested 2 times in a metabolic chamber while consuming meals with 15% or 30% protein along with beverages containing sugar sweetened beverages SSB or non-nutritive sweetened beverages (NNSB). Strengths include the study design, standardized experimental conditions, and control for the menstrual cycle in female subjects. Since the meals with SSB and NNSB were not isocaloric, the study loses some internal validity, but it has external validity because it reflects everyday beverage choices with meals. Some limitations exist, methods need clarifications in places, and the Introduction and Discussion need work. This reviewer has a few major and minor points.
MAJOR:

1. Please clarify how the four conditions were accomplished with the two visits. As written, the reader needs to deduce that the two different breakfasts and two lunches represented the four meal conditions. Did subjects have a given level of protein or a given beverage type during a given visit, or were they blended or partially blended each time? How was randomization accomplished? On a similar note, please specify how beverages were blinded.

- We have added wording to the Experimental Protocol section to expand upon this explanation (page 7, Lines 109-117).

- The look and taste of each beverage was indistinguishable. To accomplish this we used a black cherry flavor drink mix which has a strong flavor profile and we matched the sweetness of the sucralose to the sugar. Sucralose was used because it does not have the distinct, easily detected aftertastes of the other artificial sweeteners.

2. In Lines 35-38, Reference 1 is used to back a statement about 'sustaining' energy balance verses caloric balance (which are really the same thing). That was a single-meal study that showed postprandial differences in energy expenditure and substrate oxidation but not in appetite or energy intake with meals differing in macronutrient content. The main findings were with the alcohol meal. Its main conclusion is that the satiety hierarchy does not follow the oxidation hierarchy. Energy balance as the sum of macronutrient balances, as well as the oxidation hierarchy can be referenced back to the 1980's and 1990's.

- Thank you for pointing this out to us. We have removed that reference from the statement at hand and in the comment that follows in the text.

3. Similarly, in Line 45, Reference 1 is used to back up a sentence about the etiology of obesity, yet reference 1 is a single-meal study in normal weight subjects, so it cannot provide any conclusions about the etiology of obesity.

- Thank you, the reference has been deleted from the statement.

4. The test meals are cleverly designed by using the same foods but in different ways. Thus, not only are macronutrients well-controlled, but so are sub-types of macronutrients such as fibers & sugars, proteins (i.e. quality), and fatty acids (i.e. chain length & saturation). This can be listed as a strength. However, the authors should acknowledge that the main carbohydrate sources are white potatoes and white bread, which are low-fiber, processed, high glycemic index. We do not know if similar results would have been found with different carbohydrate types. The authors should discuss this, but can also point to external validity, since so many people in US and many other developed nations often chose such carbohydrate types.

- Thank you. We have added these thoughts to a paragraph stating the strengths and limitations of the study (page 24, line 478).
5. Lines 353-355: This sentence states that appetite and food preferences are significantly altered by addition of SSB to a meal. However, lines 262-265 (Results) say that there is no effect of beverage type on appetite. Lines 276-278 show that the only food preference result is the protein interaction with NNSB. Thus, findings seem to indicate that the macronutrient manipulations impacted the energy expenditure side of the energy balance equation more than the energy intake side, much like findings in reference 1.

- Thank you for the observation. We have adjusted the wording in the opening paragraph of the discussion to reflect the reviewer’s comments (page 21, lines 398).

6. Line 356-357; discussion focus on the reduction in fat oxidation without mentioning the compensatory rise in carbohydrate oxidation or increase in 24 hour EE. The full context of substrate oxidation should be discussed. This shift in substrate oxidation is not surprising. The reciprocal nature of carbohydrate and fat oxidation has been known since the Randle hypothesis in the early 1960's. The hierarchy of fuel utilization was characterized by the work of Flatt, Ravussin and others in the 1980's and 1990's, and is corroborated in reference 1. This study applies these concepts to meals differing in protein content that are consumed with SSB or NNSB. This needs to be brought forth in the Discussion.

- We have added a discussion on the reciprocal relationship between carbohydrate and fat oxidation (page 23, lines 461 – 466).

7. Lines 1-2; 36-38; 430-431: It should be clarified that this study, in the context of myriad others, demonstrates that macronutrient intake can impact both sides of the energy balance equation - intake and expenditure. That is why macronutrients matter. The authors allude to this, in part, in line 64. However, in parts of the manuscript listed above, readers get a sense that the authors are stating that it must be one or the other. This could lead to reader, and even public, confusion, so it should be clarified. It think what the authors are trying to say is that for people trying to lose weight, macronutrient selection can impact appetite and macronutrient partitioning, so it is important in helping to achieve negative energy balance. However, since these were single test meals in healthy-weight young adults, authors should use caution in extrapolating to long-term weight loss in obese individuals.

- The reviewer’s point is well taken. We have changed the discussion wording to reflect the dynamic relationship between macronutrient composition and energy balance.

MINOR:

1. Lines 35-38: The sentence starts with "Recent" yet this reference is 14 years old. As mentioned, it does not have to be recent, since the concept of macronutrient balances and differential impacts on energy intake and energy expenditure have been around for decades

- We thank the reviewer for their comment and have removed the word.
2. In Line 40, please provide the years for NHANES 1 so that readers can more readily compare that to the data you cite for NHANES 2011-2012.

- The years that NHANES 1 covers have been added (page 4, line 40).

3. Results are presented for 27 subjects. Was this the number who consented and were randomized? Were there any drops? If so, then please explain.

- There were a total of 34 participants who signed the consent. We had added this information to the manuscript (page 6, lines 85-86).

4. Was there a lower limit for BMI screening criteria?

- Participants had to have a BMI of at least 18. This lower limit for BMI has been added (page 6, line 84).

5. What was the fitness level and/or habitual activity of the subjects? Since this can influence both appetite and substrate oxidation, it is important information.

- We did not have participants wear an activity monitor during the study. We did, however, ask participants to complete a questionnaire about their physical activity for the 7 days prior to going into the calorimeter. The results from the questionnaires have been added to the manuscript (page 15, lines 288-291).

6. METHODS Lines 94-99: This section on food diaries should come after the protocol overview, so that readers are oriented first.

- Thank you for the suggestion. We have moved this section to the suggested place (page 10, line 164).

7. Line 99: Reference 17 is for the USDA database, but what is the "Customized in-house nutrient analysis program"?

- We have added an explanation about the program (page 10, lines 169-173).

8. Line 107: How long did subjects fast before blood glucose was measured?

- This was an overnight fast of at least 12 hours. This has been added to the manuscript (page 7, line 105).

9. Why was a range of 35-40 minutes used for measuring RMR?

- Our criteria for a valid RMR is a minimum of 20 min of steady state as determined by a <10% fluctuation in oxygen consumption and <5% fluctuation in respiratory quotient. This wording has been added to the Experimental Protocol section (page 8, lines 140-142).
10. Were urine samples batched for freezing? Were they measured at the same time? If so, then did storage time differ?

- Urine samples were frozen immediately after each collection period. Frozen samples were then analyzed in batches of 18 – 24 samples.

11. Line 197: Change "was" to "were" (the word 'data' is plural).

- Thank you for bringing this oversight to our attention.

12. Lines 231 and 240-241: The term 'postprandial thermogenesis' seems to be used synonymously with DIT. While some readers will understand that they mean the same thing in this case, not all will. Therefore, chose one term for clarity and consistency.

- We have changed the wording throughout the manuscript to be consistent.

13. Line 250 & 260: participants' (plural possessive)

- Thank you for bringing this oversight to our attention.

14. Line 254: Usually results for the primary outcome are presented first, yet this section begins with appetite day. The primary outcome is discussed last (line 300), yet the reader will look for it first.

- Thank you for the suggestion. We have rearranged the results and placed the primary outcome data at the beginning of the section.

15. Line 294: The acronym "DIT" was already defined, so the term does not need to be fully written out here.

- We have adjusted the wording accordingly.

16. Line 305: Do the 8% and 11% here represent the meals with the two different protein levels? If so, then please specify.

- As written, SSB consumption decreased fat oxidation by 8% ± 11%. This is the average decrease across all study parameters. We have replaced this statement and separated out the effects of the two different protein levels (page 16, line 297).

17. The higher %DIT with NNSB than SSB probably results from the fact that 500 kcal meals (NNSB) were higher in %protein than the corresponding 620 kcal meals (SSB), and protein induces the highest DIT.

- Thank you for your comment. We agree with the reviewer, however, the primary point was to show that the alteration in DIT was greater with the protein-rich meal compared to the
standard protein meal. We have reworded this statement to clarify our findings (page 23, line 444-445).

18. Lines 350-353: The Discussion's first sentence refers to SSB's and the second sentence refers to the meals' different protein contents. Something is needed between these two sentences to pull in the protein concept.

- We have reworded the first paragraph of the discussion to reflect both SSB consumption and protein intake (page 21, lines 398).

19. Line 405: provide for the readers the actual numbers of the additional 120 beverage calories that were not expended.

- We have added this to the opening paragraph of the discussion (page 21, line 405).