Reviewer’s report

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

Version: 0 Date: 14 Feb 2017

Reviewer: Kathleen Melanson

Reviewer’s report:

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.

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.

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.

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.

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.

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.

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.

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.

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.

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

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.

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

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

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

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

10. Were urine samples batched for freezing? Were they measured at the same time? If so, then did storage time differ?

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

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.

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

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.

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

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

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.

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.

19. Line 405: provide for the readers the actual numbers of the additional 120 beverage calories that were not expended.
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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Not relevant to this manuscript

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