Author’s response to reviews

Title: The effects of increased dietary protein yogurt snack in the afternoon on appetite control and eating initiation in healthy women

Authors:

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Author’s response to reviews: see over
We would like to request that the following manuscript entitled “The effects of increased dietary protein yogurt snack in the afternoon on appetite control and eating initiation in healthy women” written by Laura C. Ortinau, Julie M Culp, Heather A. Hoertel, Steve M. Douglas, Heather J. Leidy to be re-evaluated for publication as a Short Reports in the Nutrition Journal. We have responded to each of the reviewers’ comments and have revised the manuscript accordingly. All revisions are highlighted in the text, and a point-by-point description of these changes can be seen below.

The revised manuscript has been approved by all authors. Further, they have taken due care to ensure the integrity of their work and their personal scientific reputation. Lastly, the authors have not published or submitted any related papers from the same study.

We look forward to hearing from you. Thank you for your time and consideration. For correspondence concerning the manuscript please contact:

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We would like to thank the editor and reviewers for the time spent in reviewing this manuscript and providing insightful feedback. Please see our comments below which address each concern.

Reviewer: Mark Kern (MK)

Reviewer MK Overall Comments:
The manuscript is well-written and the design and interpretation of the study are clear. The results indicate that yogurts produced very similar responses, which is of potential importance given the rise in popularity of Greek yogurt. Added analyses provide information about the factors that lead to subsequent intake and these data add to the novelty of the manuscript and may provide readers with data that can be used for future projects related to hunger thresholds. The study appears to have been well-controlled and the number of subjects is quite strong. Furthermore, given the remarkable similarities between the trials, it appears unlikely that the null findings are due to a limitation in the sample size.

Authors’ Response:
We want to sincerely thank you for the kind words regarding the design, strong sample size, and novelties of the study. It is most appreciated.

Reviewer: Vicky Drapeau (VD)

Reviewer VD Overall Comment:
The objectives of this well design study (randomized cross-over design) are quite interesting and merits attention particularly in the context of the high obesity prevalence. I only have minor comments/suggestions to address to the authors.

Authors’ Response:
We want to sincerely thank you for the kind words regarding the design of the study and meaningful/important data. It is most appreciated.
Reviewer VD minor comment 1:
Background, 3rd para: The authors indicate that the purpose of this study was to evaluate the impact of a high protein snack on appetite control, satiety and delays subsequent eating compare to an isocaloric normal protein snack. In this objective, it’s seems like appetite control is separate from satiety (which is not the case). Moreover, why only indicate delays subsequent intake and not subsequent energy intake? In my opinion, after reading this study, it would be more appropriate to indicate that the main aimed of this was to evaluate the impact of high protein snack on appetite control which include satiation and satiety (assessed with visual analogue scale), delays subsequent eating (hour) as well as subsequent energy intake (kcal). This should be revised throughout the text (including the abstract).

Authors’ Response:
We appreciate the reviewer’s attention to detail, and have made the appropriate changes within the text of the manuscript.

Reviewer VD minor comment 2a:
Methods, 1st para: The authors indicate that the subjects had no eating disorders, diabetes or rapid weight fluctuation during the past six months. Is it possible to specify how eating disorders and diabetes were assessed? Please specify what was the body weight fluctuation interval, which correspond to “no rapid weight loss/gain”.

Authors’ Response:
A medical history questionnaire was used as a screening tool to identify clinically-diagnosed eating disorders and diabetes. Further, the questionnaire asked whether the participant had experienced rapid weight loss/gain (≥10 lbs) in the past six months and the potential reasons surrounding this weight loss/gain. We have included this information in the methods’ section.

Reviewer VD minor comment 2b:
In the same para, please specify if cognitive restriction and menstrual cycle was assessed in this group of subjects. Because cognitive restriction is usually high in this group of participants and that this eating behaviour trait can influence appetite control, the impact of cognitive restriction on the present study results should be addressed in the discussion (ex. Outcome on the results, limitation, etc). The same is true for the menstrual cycle and appetite control.
Authors’ Response:
We recognize that lack of control for dietary restraint might be a limitation of this study. As stated above, we did, however, screen out those who were clinically diagnosed with an eating disorder and those that had atypical eating behaviors and/or patterns (1 meal/day; 6 meals/day; vegetarian; high protein, etc.), or those that never or infrequently snacked. We sought to include participants that would be representative of a healthy female population albeit potentially containing restrained eaters.

With respect to menstrual cycle, there is conflicting and limited data as to the extent that menstrual cycle phase influences acute appetite control and food intake [1-4]. Recent data from our laboratory suggests that there is no effect of menstrual cycle on acute appetite control and food intake [5]. Regardless, due to the limited time frame of the current study, we were unable to schedule each participant’s testing days during the follicular phase. However, by randomizing the order of the snack conditions between subjects, it is likely that we had an equal distribution of testing days that fell in the follicular and/or luteal menstrual phases.

We have included the lack of control for dietary restraint and menstrual cycle in the ‘Study Limitations’ section of the manuscript.

Reviewer VD minor comment 3a:
Methods, experimental design: Please specify which visual analogue scale was used – 100 mm or 150 mm?

Authors’ Response:
This was a 100 mm VAS. We have added this information to the Methods’ section.

Reviewer VD minor comment 3b:
Also specify if the participants were allowed to do some activities between in the 8 h testing period (ex. Reading, watching TV, etc). In my opinion, 8 hour testing is very long for a participants and the dinner could have been request because they were bored.

Authors’ Response:
Although the 8 hour testing day is very long, the participants were permitted to relax and engage in numerous (sedentary) activities. Some of these include reading, watching movies, homework, etc. Many of the participants actually expressed that they looked forward to the testing days as a way to relax and do some ‘enjoyment/fun’ reading or get caught up on work. We have added this information to the Method’s section.
Reviewer VD minor comment 4:
Methods experimental design: The authors indicate that they evaluate perceived appetite: hunger, desire to eat and prospective food consumption) and satiety (fullness). This is a little bit unclear as fullness is part of all perceived appetite sensations and can either assessed satiation (i.e. the termination of a meal) or satiety (i.e. the period of time between two eating episodes). The same is true for each appetite sensation. It should be adjusted in the text.

Authors’ Response:
In the past, our research group as well as others in the field, have used the term ‘appetite’ to indicate those sensations (or responses) that drive the initiation of eating, whereas ‘satiety’ is an indicator of fullness (and the sensation to prevent eating initiation). Regardless, we will modify the terminology in the text to reflect ‘perceived sensations’ of hunger, fullness, and desire to eat.

Reviewer VD minor comment 5:
Data statistical analysis: Please specify if Person correlations were performed on one appetite sensation time point or on changes in appetite sensation in response to the snack or during afternoon.

Authors’ Response:
Pearson correlation analyses were performed on each post-snack time point for the following continuous variables: perceived hunger, fullness, desire to eat, and actual time of day as well as the categorical variable, habitual dinner time, which was collected from screening food records. For the later variable, at each time point, we enter ‘Yes’ this is the habitual time in which the participant typically eats dinner or ‘No’ this is not the habitual time in which the participant typically eats dinner. These variables were correlated with another categorical variable, time to dinner request. For this variable, at each time point, we entered either ‘Yes’ dinner was requested or ‘No’ dinner was not requested. This information has been added to the data and statistical analysis section.

Reviewer VD minor comment 6:
Discussion, 2 para: In line with comment no. 4, this sentence should be adjusted “In addition to changes in perceived sensations of fullness over time, another ....”

Authors’ Response:
This has been revised accordingly.
Reviewer VD minor comment 7:
Discussion: Please discuss briefly some limitations (specific population, women, etc).

Authors’ Response: Due to the tight word limit with a short communications (1500 words) we previously omitted the limitations section. However, we agree with the reviewer that this needs to be included and have revised accordingly.

Reviewer VD minor comment 8:
Abbreviations: Why – NS for No snack? no mention in the text.

Authors’ Response: We apologize for this typo and have removed it from the text.

Reviewer VD minor comment 9:
Figure 1: Please add indications regarding lunch and snack consumption. The post snack area under the curve units should be (mm * 120 min) since the first post snack perceived appetite sensation was taken 30 min after the snack.

Authors’ Response: We apologize for these errors/omissions and have made the appropriate revisions to the figure.

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Reviewer: Didier Chapelot

Reviewer DC General Comment 1:
The procedure fulfills the multidimensional evaluation of satiety (assessment of intensity, duration and further intake) considered by the reviewer as the best approach of satiety to this day. The sample size (n = 32) is rather high compared to other experiments in this domain and represents one of its strengths.

Authors’ Response: We want to sincerely thank you for the kind words regarding the design and strong sample size of the study. It is most appreciated.

Reviewer DC General Comment 2:
The absence of a control condition could be considered as a weakness (precluding to infer whether the snack alters satiety or not) but does not compromise answering to the initial hypothesis.
Authors’ response:
We sought to test if 14 g of protein in a 160 kcal afternoon yogurt snack would be adequate to elicit protein-related alterations in appetite control and satiety compared to a commonly consumed, isocaloric afternoon yogurt snack containing 5 g of protein (i.e., which is the typical protein quantity in this food form in the U.S.). The participants in this study were habitual, afternoon snack consumers, who frequently consumed the 5 g yogurt snack. Thus, the 5 g yogurt served as the control condition.

Reviewer DC General Comment 3:
The manuscript is unfortunately quite short, several informations lacking (see below), and even if no differences were observed between the two experimental snacks, more details would have been needed. The discussion is in particular very poor.

Authors’ response
We appreciate the comments pertaining to the abbreviated information throughout the paper. This manuscript was submitted as a ‘short communication’ with only 1500 words permitted for the entire manuscript, including the discussion (along with only 25 references). Because of this we chose to briefly describe the materials and methods as well as briefly discuss/compare the findings in order to adhere to the word limit of a ‘short communication’. (We will address specific comments within each section below.)

Reviewer DC Major Compulsory Revision 1a:
Why were subjects allowed to request their dinner only when scales were filled, leading to a 30 min fixed delay between conditions? This seems relatively hard to explain given such a procedure artificially reduces the sensitivity of the measure (most of differences in durations are lower than 30 min).

Authors response:
Participants were allowed to request to eat at any time. Specifically, at the beginning of each testing day, the research staff informed the participants that we would periodically ask whether they were ready to eat again (after the snack); however, if they wanted to eat outside of the times in which we ask them this question, they could simply let us know. (Several of the participants did, in fact, request to eat outside of these times.)
We have added these details to the Methods’ section.

Regarding the VAS questionnaire, participants were given random as well as incremental (every 30 min) VAS to complete throughout the testing day. Only the 30 min time intervals (for indices of appetite and satiety) were entered and reported. Therefore, the sensitivity of our measurement was not reduced by the experimental design. These
details have also been added to the experimental design. Additionally, previous snack studies have used this experimental design and were used for power calculations of this outcome [6-8]. However, further clarifications of these procedures have been included in the methods.

Reviewer DC Major Compulsory Revision 1b:
References cited do not consist in studies showing that this question provided with VAS every 30 min is appropriate to determine duration of satiety but only that VAS scores are reliable for assessing satiety.

Authors’ Response:
See previous comment above. Additionally, along with including the Marmonier et al. 2000 citation within the statistical analysis section of the manuscript, we have added 2 other citations to support that time to meal request is a reliable measure of satiety. See refs. below.
(Marmonier et al. 1999; Marmonier et al. 2002) in text [23-24].

Reviewer DC Major Compulsory Revision 2a:
The statistical analysis plan should be clarified and improved. Why no ANOVA for repeated measures were conducted and only paired t-tests for comparing AUCs? The two are complementary and the former may reveal some transient differences that the single-value AUC result may miss.

Authors’ Response:
We appreciate the comment and have re-analyzed the data to include the repeated measures ANOVA to determine main effects of time, condition, and time x condition interactions as well as the paired t-tests.

Reviewer DC Major Compulsory Revision 2b:
The analysis of VAS data derived from freely requested meals should not by definition stop at a given time-point.

Authors’ Response:
We agree and have included all VAS time points in the analyses.

Reviewer DC Major Compulsory Revision 2b:
Although this delay was apparently very similar between conditions and across subjects (2:43±0:06 and 2:41±0.04 h), a retrograde analysis would have added some information on the motivation to eat over the preprandial phase.
Authors’ Response:
A retrograde analysis had been previously performed (see below).

Since no differences were found between conditions (and due to word limitations of the short communications), we chose to eliminate this from the analyses, results, and discussion. If this reviewer feels this information is critical to the paper, we would be happy to include in the manuscript (should be get approval from the editors to do so).

Reviewer DC Major Compulsory Revision 2b:
Furthermore, if some subjects have requested their meal 120 min following the consumption of a snack, the number of subjects is not 32 at 150 min in one or both conditions. Please specify.

Authors’ Response:
You are correct in that not all volunteers had 150 and 180 min time points. We have included this information in the subheading of the Figures.
Reviewer DC Major Compulsory Revision 2b:
Last, why differences in time to dinner request between conditions were not modelized in the multilinear regression? Or is the difference was actually the dependent variable of the regression in the present analyses? Actually, the predictors entered in the model are not clear for the reviewer.

Authors’ Response:
We first want to acknowledge that we worked with a Biostatistician, Dr. Youngju Pak, at the University of Missouri on the most appropriate statistical approach to answering the research question related to ‘key factors that predict the onset of eating.’ Based on Dr. Pak’s recommendations, Pearson correlation analyses were performed on each post-snack time point for the following continuous variables: perceived hunger, fullness, desire to eat, and actual time of day as well as the categorical variable, habitual dinner time, which was collected from screening food records. For the later variable, at each time point, we enter ‘Yes’ this is the habitual time in which the participant typically eats dinner or ‘No’ this is not the habitual time in which the participant typically eats dinner. These variables were correlated with another categorical variable, time to dinner request. For this variable, at each time point, we entered either ‘Yes’ dinner was requested or ‘No’ dinner was not requested. This information has been added to the data and statistical analysis section. A multivariate regression was then performed using the previously stated variables found to be associated with time to dinner request. Note: we initially performed separate multi-linear regressions for each study condition. However, because the analyses were similar, we chose to combine both conditions and report overall findings in the paper.

Reviewer DC Major Compulsory Revision 3:
I disagree with authors on the last paragraph of their discussion. Perceived hunger should not replace spontaneous meal request because they found a significant (and very weak) correlation between these two variables, and 80 mm should not be considered as a universal threshold for meal triggering. A long experience in the domain shows that there is a large interindividual variability in the way the space of these scales (actually horizontal lines) is used by subjects and that a significant proportion of them reach 80 mm long before asking for their meal and only ask it in the 90-100 mm interval. To establish 80 as a hunger threshold, authors should have done complementary statistics and analyses of individual data.

Authors’ Response:
We appreciate your comment and acknowledge your expertise in this field. However, very few studies have been completed which allow for the examination of predictors of
eating initiation. Thus, although we feel the data and subsequent findings are meaningful, relevant, and accurate, we have revised the last paragraph to emphasis that the findings are not necessarily generalizable to other populations, etc.

**Reviewer DC Minor Essential Revision 1:**
Method – Participants. How authors managed the putative usual ovulatory cycle effect on their results?

**Author’s Response**

With respect to menstrual cycle, there is conflicting and limited data as to the extent that menstrual cycle phase influences acute appetite control and food intake [1-4]. Recent data from our laboratory suggests that there is no effect of menstrual cycle on acute appetite control and food intake [5]. Regardless, due to the limited time frame of the current study, we were unable to schedule each participant’s testing days during the follicular phase. However, by randomizing the order of the snack conditions between subjects, it is likely that we had an equal distribution of testing days that fell in the follicular and/or luteal menstrual phases. We have addressed these concerns in the Limitations’ section.

**Reviewer DC Minor Essential Revision 2:**
How the absence of eating disorders was assessed? And was there any estimation of the subjects’ restrained eating status given the pivotal role of this psychic trait in spontaneous eating behaviour?

**Authors’ Response:**
The absence of eating disorders was assessed through a medical history questionnaire provided to potential participants prior to enrollment in the study. Participants were asked specifically if they had been previously diagnosed with any eating disorders.

Dietary restraint was not specifically assessed and we recognize that it might be a limitation of this study. We did, however, screen out those who were clinically diagnosed with an eating disorder, those that displayed rapid weight gain and/or loss over a short amount of time (6 months or less), those that had a-typical eating behaviors and/or patterns (1 meal/day; 6 meals/day; vegetarian; high protein, etc.), or those that never or infrequently snacked. We sought to include a sample size that would be representative of a healthy female population albeit potentially containing restrained eaters. This has also been added to the Limitations’ section.
Reviewer DC Minor Essential Revision 3:
Was there any attempt to match sensory characters of the two snacks? And was this evaluated?

Authors’ Response:
No, we chose not to match sensory characteristics of the two snacks since this was a practical comparison of two commonly consumed yogurt snacks found in the grocery stores. However, sensory characteristics were evaluated. We previously included palatability of the snacks in Table 1 but have now included all sensory characteristics assessed as well as the statistical analyses.

Reviewer DC Minor Essential Revision 4:
Method – Experimental design. Why do authors chose a fixed lunch energy load (500 kcal for all subjects)? Thanks, justify this approach compared to alternative ones (energy – body weight ratio, percentage of energy requirements, usual energy intake at this meal...)

Authors’ Response:
We recognize that other methods such as energy/body weight ratio, percentage of energy requirements, and usual energy intake at a meal are also acceptable experimental methods. We chose a fixed energy content lunch for several reasons. According to the NHANES data, the energy content of lunch consumed by US adults is 500, which is approximately 24% of daily intake [9, 10]. Thus, we chose a 500 kcal lunch which would be representative of most US Americans (in terms of percentage of energy requirements AND usual energy intake at that meal). We have also used this approach in previous published studies with success [5, 11].

Reviewer DC Minor Essential Revision 5:
Methods-Please specify the type of scales used for palatability assessment.

Authors’ Response
Snack palatability (i.e., appearance, aroma, flavor texture and overall liking) were assessed for each snack pattern using 100 mm visual analogue scales (VAS). The questions were worded as “how strong is the... or how much do you like the...” with anchors at extremely dislike/low or extremely like/high. This information has been included.
Reviewer DC Minor Essential Revision 6:
Methods-Was there any kind of dietary counseling for matching diets on days prior to each test session?

Authors’ Response
Prior to the start of the study, the volunteers recorded their habitual breakfast, lunch, and afternoon snack times during screening through food frequency questionnaires which included documentation of how often, what type, and what time of day the participants consumed meals and snacks in the past month. During the 3 acclimation days (proceeding each testing day), the volunteers were asked to eat a specific times (breakfast: habitual time between 6 and 9 am; lunch: 3 h after breakfast between 11 am and 1pm; snack: 3 h after lunch between 2 and 4 pm). We allowed dinner to vary. The participants were provided with a breakfast meal to consume the morning of testing and study snacks to consume during each of the 3 acclimation days. However, during all meals, the participants were asked to maintain habitual dietary habits.

Reviewer DC Minor Essential Revision 7:
Results-Was perceived hunger the only or the best predictor? If the answer is the latter, which other factors were also predictors?

Authors’ Response:
Hunger was the best predictor of time to dinner request. Other predictors of time to dinner request were habitual dinner time and perceived fullness. We have revised the text in the Results’ section accordingly.

Reviewer DC Minor Essential Revision 8:
Results-Energy intake prior to dinner meal was low for young women (960 kcal) and even after the ad lib dinner meal (700 kcal), only reached 1660 kcal. Authors should discuss this point.

Authors’ Response:
The study population consisted of normal to overweight women. Additionally, throughout the testing days, the women were extremely sedentary, sitting/reclining in chairs for 8h. Thus, the consumption of ~1660 kcal is adequate for this population of women throughout the study period. We also want to mention that evening intake was not assessed once the women left our facility. This would have contributed to additional calories.
Reviewer DC Minor Essential Revision 9:
Conclusion(s)-12 - The end of the last sentence is dubious. “protein-related improvements in markers of energy intake regulation” is a rather inaccurate formulation for summarizing the present results.

Authors’ Response:
We have revised accordingly.

Reviewer DC Minor Essential Revision 10:
Figure 1-“paired-sample t-tests; *P<0.05” is not necessary since there was no significant difference. Moreover, the only * symbol in the figure is for the unity (mm*150 min) and should be replaced by a point.

Authors’ Response:
We have revised accordingly.

Reviewer DC Minor Essential Revision 11:
14 - The level of motivation to eat after dinner would have been interesting to show (or evaluate), to verify whether subjects had actually eaten until satiation.

Authors’ Response:
We have included the post-dinner hunger and fullness data. See Figures 1a&b and results section.

Reviewer DC Discretionary Revision 1:
Background section-Lines 3-4. The definition of a snack as “any eating occasion outside of a typical meal time” is more a cultural than a physiological one and alternative ones are proposed (Chapelot, 2011). It would have been cautious to moderate the sentence claiming the definition of snacks.

Authors’ Response:
We appreciate the reviewers note, and acknowledge that there are many ways of defining what eating occasions are considered snacks [7, 12-22]. For our study we chose to define a snack as any eating occasion outside of a typical meal time that was self-described by participants as a snack when completing an eating frequency questionnaire during screening [14, 17, 19, 21].
Reviewer DC Discretionary Revision 2:
Background section Lines 9-10-References cited are not exactly relevant to the effects of protein on “appetite control, satiety and regulation of energy intake” but on weight loss achieved and maintained. Although this may actually result from effects of protein on appetite, satiety and energy intake (that would each need a specific definition), this is not similar. Thus, a hypothesis is that protein may actually impair appetite control and regulation of energy intake leading individuals to eat less than their energy requirement, resulting in a negative energy balance. Authors should have chosen to cite references supporting the effects of protein on appetite, satiety and intake, or to do a statement in agreement with cited references. References 11 and 12 are finally more appropriate than these ones.

Authors’ Response:
We have revised the references accordingly.

Reviewer DC Discretionary Revision 3:
Data and statistical analysis-It’s not clear why ref. 15, 16 and 18 were cited to support the power calculation and the necessary sample size of 32 since these studies involved 11, 27 and 46 subjects, respectively, and only the first included an assessment of satiety by duration. For VAS scores, a number of subjects of 18 has been proposed to detect a significantly relevant effect (de Graaf, 1993).

Authors’ Response:
The number of participants was based on power calculations performed on existing data from the referenced snack studies for several study outcomes (i.e. VAS perceived sensations and eating initiation.) Although the VAS data would lend for a sample size of n=18, the time to dinner request led to a sample size of n=32 to provide 80% power to detect differences.

Reviewer DC Discretionary Revision 4:
Method – Experimental design-Line 12. Satiety is not strictly gastric fullness but a non-hunger state and therefore should be preferentially used as the generic name for hunger, appetite, prospective consumption and gastric fullness (and other possible indices). This would allow a “common ground” terminology. It is however true that “motivation to eat” is a usual term for these various sensation ratings but rarely satiety is limited to fullness.
Authors’ Response:
We appreciate the reviewer’s suggestion; however, based on our previous publications as well as current literature using pre-load/acute meal designs, we have chosen to keep the terminology the same.

Reviewer DC Discretionary Revision 5a:
Discussion-In the Poppitt et al. study (ref. 18), the 5 g and 20 g protein preloads were beverages and not snacks per se and provided 20 and 80 kcal, respectively and not 80 and 200 kcal as written.

Authors’ Response:
We apologize for the discrepancy regarding the stated calories; this was an error on our part and has been revised accordingly.

Reviewer DC Discretionary Revision 5b:
The Chapelot & Payen study (ref. 25) could have been added in the paragraph about high versus low-protein snacks since snacks differed in protein content without being sensorally similar as in the present study (which is a strength of the latter). Moreover, the protein content of the snacks in the Chapelot & Payen study (3 vs 10 g) was more similar to this study than the (cited) Marmonier et al. even if the energy load (300 kcal) was greater.


Authors’ Response
We have included this reference accordingly.
References (pertinent to Author’s Responses):

9. Lunch: Percentages of Selected Nutrients Contributed by Foods Eaten at Lunch, by Gender and Age [www.ars.usda.gov/ba/bhnrc/fsrg]
10. Energy Intakes: Percentages of Energy from Protein, Carbohydrate, Fat and Alcohol, by Gender and Age [www.ars.usda.gov/ba/bhnrc/fsrg]


