PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	The Impact of a Powdered Meal Replacement on Metabolism and Gut Microbiota (PREMIUM) in Individuals with Excessive Body Weight: A Study Protocol for a Randomized Controlled Trial
AUTHORS	Montenegro, Julia; L. P. Oliveira, Camila; Armet, Anissa M.; Berg, Aloys; Sharma, Arya; Mereu, Laurie; Cominetti, Cristiane; Ghosh, Sunita; Richard, Caroline; Nguyen, Nguyen; Cani, Patrice; Walter, Jens; Prado, Carla

VERSION 1 – REVIEW

REVIEWER	Chambers, Edward Imperial College London Faculty of Medicine, Department of Metabolism, Digestion and Reproduction
REVIEW RETURNED	12-Dec-2022

GENERAL COMMENTS	This is a well-written study protocol that has a clearly defined objective and appropriate study design to answer the research question. The authors identify the main limitation of the study (lack of appropriate placebo/double-blinding); however this is a problem inherent to this type of dietary intervention. Specific comments: Is the use of “individuals with excessive body weight” appropriate? Individuals with overweight and obesity would be the correct clinical description of participants. Randomisation is stratified only by sex. Consequently, there is a risk that all obese volunteers will be randomised to placebo and overweight to intervention that will skew outcomes. Why have greater controls to match groups (age, BMI etc.) not been considered? The screening blood tests would not identify individuals with prediabetes/diabetes. Exclusion for abnormal glucose homeostasis would appear to be on self-reporting of a diagnosis rather than an objective study measure. Participants with diabetes could therefore be enrolled. The protocol states that a body weight change +/- 2% will be used to withdraw from study. Is there a reference to support this? <3% is normally considered weight-maintenance: https://www.nature.com/articles/0803175

REVIEWER	Mackay, Dylan University of Manitoba, Community Health Sciences
REVIEW RETURNED	14-Feb-2023

GENERAL COMMENTS	The authors have submitted a well written protocol manuscript outlining the PREMIUM RCT looking at the impact of a powdered meal replacement consumption on inflammatory markers (IL-6 as

	the primary outcome) and the gut microbiome (Secondary outcome). While the protocol is clearly written there are details related to methodology of the trial that need to be added to make the protocol manuscript prior to it being ready for publication. The issues that I think needs to be addressed are outlined below: 1)Line 121 In the outcomes there seems to be some ambiguity in the primary outcome, it says over time (within groups) and between groups, these are two separate things potentially, so it should be clearly outlined which analysis is the primary outcome, given the design has a control intervention, the primary outcome in my opinion should be the IL-6 concentration in the PMR at the end of the intervention, against the control, and the baseline values can be included in the analysis. This applies less to the exploratory outcomes, but it sort of makes 2 outcomes out of each one the way it is currently written. 2)Line 122, both the manuscript and clinical trials registry should list what measure of gut microbiome composition will be used as the secondary outcome or outcomes, but as it is currently written "Gut microbiome composition over time" is too vague. 3)Line 177, more detail should be provided around the randomization, what code is used in excel, who will be running the code, how is allocation being concealed? 4)Line 222 This line on multicomponent modeling should be expanded, at least briefly, because as written it is too vague. 5) Line 262 At least for the primary outcome, some details on what methodology is planned would be nice. Ideally the planned method for each of these outcomes should be listed. 6)Line 369 Details on the interim analysis and how the sample size would be adjusted needs to be defined. Is this an adaptive design trial? Will there be adjustment for the alpha send with the interim analysis, will the second analysis be adjusted and how, are there stopping rules for the interim analysis, what will be the methodology being used for the sample size reassessment. 7)Line 375 What is the rationale for testing for differences at baseline, this is a randomized trial so there is really no need to do testing in the baseline. Even if you have differences, that is fine if we randomized properly, if you did not then what will you do? how is it being used? See https://medium.com/peter-flom-the-blog/do-not-put-p-values-in-your-table-1-8aad0d6c92d 8)Line 392 More details, even if just general because they are not final or evolving, should be provided, especially how the Gene expression will guide the genetic polymorphism work. 9)Line 422 and line 727 Health Canada review is mentioned in 422, I assume this is because of a CTA? The retention period for clinical trial records is 15 years under the Food and Drug Regulations and Natural Health Products Regulations

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1.

This is a well-written study protocol that has a clearly defined objective and appropriate study design to answer the research question. The authors identify the main limitation of the study (lack of appropriate placebo/double-blinding); however this is a problem inherent to this type of dietary intervention. Specific comments:

1) Is the use of “individuals with excessive body weight” appropriate? Individuals with overweight and obesity would be the correct clinical description of participants.

R: We used the term to include both the overweight and obesity categories, we defined this in the text (please see lines 78-79). In Canada, it is advocated to use as people-first language. Patients prefer the term “individuals with excessive body weight” (Puhl, 2020).

Puhl, R.M., 2020. What words should we use to talk about weight? A systematic review of quantitative and qualitative studies examining preferences for weight-related terminology. *Obesity Reviews*, *21*(6), p.e13008.

2) Randomisation is stratified only by sex. Consequently, there is a risk that all obese volunteers will be randomised to placebo and overweight to intervention that will skew outcomes. Why have greater controls to match groups (age, BMI etc.) not been considered?

R: We agree, but it is unfeasible to stratify by all co-variables. In our experience, there is often overrepresentation of females in this kind of study; hence, we decided to stratify the randomization by sex (categorical variable) to ensure equal distribution of sex between groups. We expect to have participants across all BMI and age ranges (as continuous variables), which, along with the randomization itself, minimizes the risk of overrepresentation in each group.

3) The screening blood tests would not identify individuals with prediabetes/diabetes. Exclusion for abnormal glucose homeostasis would appear to be on self-reporting of a diagnosis rather than an objective study measure. Participants with diabetes could therefore be enrolled.

R: Indeed, the diagnosis of diabetes or other diseases is self-reported. If the person has symptoms or use any medication for diabetes or other chronic diseases, they are ineligible. This has been clarified in the text (please see lines 192-195)

4) The protocol states that a body weight change +/- 2% will be used to withdraw from study. Is there a reference to support this? <3% is normally considered weight-maintenance: https://www.nature.com/articles/0803175

R: We used 2% change in body weight to determine the consultation with the registered dietitian to avoid getting to a significant weight change (i.e., 3%). The discontinuation criteria has been updated (please see line 390). Notably, no participants to date have presented with such substantial weight change.

Reviewer: 2.

The authors have submitted a well written protocol manuscript outlining the PREMIUM RCT looking at the impact of a powdered meal replacement consumption on inflammatory markers (IL-6 as the primary outcome) and the gut microbiome (Secondary outcome). While the protocol is clearly written there are details related to methodology of the trial that need to be added to make the protocol manuscript prior to it being ready for publication. The issues that I think needs to be addressed are outlined below:

1) Line 121 In the outcomes there seems to be some ambiguity in the primary outcome, it says over time (within groups) and between groups, these are two separate things potentially, so it should be

clearly outlined which analysis is the primary outcome, given the design has a control intervention, the primary outcome in my opinion should be the IL-6 concentration in the PMR at the end of the intervention, against the control, and the baseline values can be included in the analysis. This applies less to the exploratory outcomes, but it sort of makes 2 outcomes out of each one the way it is currently written.

R: The strength of the randomized controlled trial design lies in its ability to facilitate comparisons both between time points and between groups. By employing suitable statistical methods, such as two-way mixed ANOVA and Generalized Estimated Equations, the study will concurrently assess time (interindividual) and group effects.

2) Line 122, both the manuscript and clinical trials registry should list what measure of gut microbiome composition will be used as the secondary outcome or outcomes, but as it is currently written “Gut microbiome composition over time” is too vague.

R: Additional details have been included (please see lines 137-140).

3) Line 177, more detail should be provided around the randomization, what code is used in excel, who will be running the code, how is allocation being concealed?

R: Additional details have been included (please see lines 198-201).

4) Line 222 This line on multicomponent modeling should be expanded, at least briefly, because as written it is too vague.

R: We included the components of the 4-compartment model and the method to assess each compartment (please see lines 249-250).

5) Line 262 At least for the primary outcome, some details on what methodology is planned would be nice. Ideally the planned method for each of these outcomes should be listed.

R: We included the methods for blood analysis (please see lines 290-292).

6) Line 369 Details on the interim analysis and how the sample size would be adjusted needs to be defined. Is this an adaptive design trial? Will there be adjustment for the alpha send with the interim analysis, will the second analysis be adjusted and how, are there stopping rules for the interim analysis, what will be the methodology being used for the sample size reassessment.

R: This trial is not adaptive; the post-hoc decision was made in response to challenges arising from the COVID-19 pandemic, such as higher drop-out rates than anticipated and delayed recruitment. Notably, the interim analysis's primary purpose was to ensure sample quality, rather than adjusting the sample size. Given the delays in recruitment and the ongoing pandemic, we recently considered the need to analyze samples midway through the study due to the potential degradation of reagents and equipment over time. A sample size reassessment was not the objective, so it has been removed from the protocol (please see line 407-408).

7) Line 375 What is the rationale for testing for differences at baseline, this is a randomized trial so there is really no need to do testing in the baseline. Even if you have differences, that is fine if we

randomized properly, if you did not then what will you do? how is it being used? See https://medium.com/peter-flom-the-blog/do-not-put-p-values-in-your-table-1-8aad0d6c92d

R: The reviewer is correct, we removed it from the text (please see lines 413-414).

8) Line 392 More details, even if just general because they are not final or evolving, should be provided, especially how the Gene expression will guide the genetic polymorphism work.

R: We included further details of gene expression and genetic polymorphism methodology (please see lines 426-440). We also included more details in the experimental analysis of gene expression and genetic polymorphism (please see lines 311-329).

9) Line 422 and line 727 Health Canada review is mentioned in 422, I assume this is because of a CTA? The retention period for clinical trial records is 15 years under the Food and Drug Regulations and Natural Health Products Regulations

R: We corrected in the text (please see line 474)

VERSION 2 – REVIEW

REVIEWER	Chambers, Edward Imperial College London Faculty of Medicine, Department of Metabolism, Digestion and Reproduction
REVIEW RETURNED	21-Jun-2023

GENERAL COMMENTS	All comments have been addressed in the revised manuscript.

REVIEWER	Mackay, Dylan University of Manitoba, Community Health Sciences
REVIEW RETURNED	13-Jul-2023

GENERAL COMMENTS	The authors have done an excellent job in responding to the previous comments, however there is still some things that should be clarified prioir to publication that I think will greatly improve the protocol manuscript. The biggest is the ambiguity in the primary outcome (and secondary outcomes), and other outcomes are written, from the protocol manuscript and response to comments I believe the authors are interested in the time by treatment effect as the primary outcome, this should just be explicitly stated so it is clear, as written "outcome is to compare changes in IL-6 concentration over time (within groups) and between the PMR and CON groups" could simply be written as "outcome is to compare changes in IL-6 concentration over time (within groups) between the PMR and CON groups". removing the "and" in this line and others like it makes this more clear. The other big thing that I think should be highlighted in the strengths and limitations section is the limitation of the sample size (n=74) in relation to the planned multi-omics work, especially the nutrigenetic work, this needs to be explicitly framed as exploratory and hypothesis generating. Without this strong framing any statistically significant findings from this exploratory work risks being overinterpreted when they will most likely be due to chance dichotomization.

	Additional minor things to change include: 1.When describing the secondary outcome, it should really be "secondary outcomes" as at least 7 different diversity indexes, as well as numerous other ways of looking at the gut microbiome are listed in the protocol. Given this large a number I would recommend picking a shorter list of the preferred secondary outcomes and moving the bulk of the gut microbiome analysis to exploratory outcomes as well. If you list too many outcomes it reduces the point of having a hierarchy of outcomes. 2. detailing exactly how the randomization was conducted in excel, as in what commands were used, would improve clarify and transparency, as written in the expanded section it is still not clear. I am guessing from the text that a researcher created 4 lists of random numbers (how those random numbers were created is not clear, I guess the Rand() function?) and then another researcher followed that list, was there any allocation blinding, did the next researcher who did the allocation get the assignment one at a time (like a sealed envelop or via a computer application, or from the creator of the order) at the time of randomization or could they see the entire list?

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1.

All comments have been addressed in the revised manuscript.

R: Thank you.

Reviewer: 2.

The authors have done an excellent job in responding to the previous comments, however there is still some things that should be clarified prior to publication that I think will greatly improve the protocol manuscript.

R: Thank you for the further suggestions that helped us improve the manuscript.

1. The biggest is the ambiguity in the primary outcome (and secondary outcomes), and other outcomes are written, from the protocol manuscript and response to comments I believe the authors are interested in the time by treatment effect as the primary outcome, this should just be explicitly stated so it is clear, as written "outcome is to compare changes in IL-6 concentration over time (within groups) and between the PMR and CON groups" could simply be written as "outcome is to compare changes in IL-6 concentration over time (within groups) between the PMR and CON groups". removing the "and" in this line and others like it makes this more clear.

R: We have incorporated this suggestion (please see lines 125-151).

2. The other big thing that I think should be highlighted in the strengths and limitations section is the limitation of the sample size (n=74) in relation to the planned multi-omics work, especially the nutrigenetic work, this needs to be explicitly framed as exploratory and hypothesis generating. Without

this strong framing any statistically significant findings from this exploratory work risks being overinterpreted when they will most likely be due to chance dichotomization.

R: We reframed the strength of the multi-omics approach (please see lines 55-58).

3. When describing the secondary outcome, it should really be "secondary outcomes" as at least 7 different diversity indexes, as well as numerous other ways of looking at the gut microbiome are listed in the protocol. Given this large a number I would recommend picking a shorter list of the preferred secondary outcomes and moving the bulk of the gut microbiome analysis to exploratory outcomes as well. If you list too many outcomes it reduces the point of having a hierarchy of outcomes.

R: We included relative abundances of amplicon sequence variant (ASV) as the secondary outcome, and the remaining gut microbiome diversity indexes and taxonomic assignments are now exploratory outcomes (please see lines 126-134).

4. Detailing exactly how the randomization was conducted in excel, as in what commands were used, would improve clarify and transparency, as written in the expanded section it is still not clear. I am guessing from the text that a researcher created 4 lists of random numbers (how those random numbers were created is not clear, I guess the Rand() function?) and then another researcher followed that list, was there any allocation blinding, did the next researcher who did the allocation get the assignment one at a time (like a sealed envelop or via a computer application, or from the creator of the order) at the time of randomization or could they see the entire list?

R: The website Randomization.com (http://www.jerrydallal.com/random/randomize.htm) was used to generate two separate random allocation sequences (female and male sequences). A second investigator follows the predetermined order of numbers and assigns participants to their respective groups based on the order of their screening. The list of random numbers is covered and the investigator assigning participants to their respective group does not look at the numbers; however, the investigator has access to it (not blinded). We included this in the text (please see lines 193-199).

VERSION 3 – REVIEW

REVIEWER	Mackay, Dylan University of Manitoba, Community Health Sciences
REVIEW RETURNED	22-Aug-2023

GENERAL COMMENTS	The authors have done an excellent job of addressing all of the final recommendations.