Author’s response to reviews

Title: Effect of whey protein supplementation on levels of endocannabinoids and some of metabolic risk factors in obese women on a weight-loss diet: A study protocol for a randomized controlled trial

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Reviewer #1:

- The authors aimed to create a study protocol showing the effects of whey protein on endocannabinoids plasma levels in obese women. The study protocol has some impact to those with closely related research interests. It is well known that high protein levels consumption can overload some tissues such as kidney, especially in absence of physical exercise. Authors should discuss the "negative impacts" of whey protein on cardiorenal system.

Recent trends in weight loss diets have led to a substantial increase in protein intake by individuals. As a result, the safety of habitually consuming dietary protein in excess of recommended intakes has been questioned. In particular, there is concern that high protein intake may promote renal damage by chronically increasing glomerular pressure and hyperfiltration. In fact, some studies suggest that hyperfiltration, the purported mechanism for renal damage, is a normal adaptive mechanism that occurs in response to several physiological conditions. Although excessive protein intake remains a health concern in individuals with pre-existing renal disease, the literature lacks significant research demonstrating a link between protein intake and the initiation or progression of renal disease in healthy individuals.

The study subjects in this trial are healthy and non-menopause women, and we will mention that drink abundant water after consumption of protein powder.

- We know that researches usually are followed by problems and pitfalls, in this case what would the possible pitfalls be in the present research? How about their solutions to solve them?
The possible pitfalls and problems in the present research will be: - To calculate the energy requirements and macronutrient distribution.
- Creating a variety in the diet while maintaining the general principles of diet.
- Checking the regular consumption of protein powders.

And, the solutions to solve them, respectively;

- A trained dietitian will design the energy requirements and macronutrient distribution and the same dietitian will be available to train participants on the diet.

- Subjects will be asked to record powder intake and to check compliance, they will be followed by the dietitian via phone calls or SMS, every three days.

- To create a variety in the diet while maintaining the general principles of diet, the same dietitian will give a dietary exchange list and a diet according to their food habits.

- Please review the text. Some points the reader cannot interpret it well. “Epidemiological studies indicate that the consumption of milk and dairy products is inversely associated with a lower risk of metabolic disorders and cardiovascular diseases” what did the authors mean with this sentence? Is milk bad or good for metabolic and cardiovascular dysfunctions?

We reviewed the text and corrected this sentence. (Epidemiological studies indicate that the consumption of milk and dairy products is associated with a lower risk of metabolic disorders and cardiovascular diseases).

Reviewer #2:

- The proposed research is interesting, and the manuscript is clear and well written.

However, the details on the diet protocol is scarce. What type of diet guide will be provided to participants? It is important to demonstrate participant compliance to the weight loss diet in the first few weeks of the study as there will be a learning curve for participants. Will there be a dietitian available to train participants on the diet? Will any foods be provided to participants to ease participant burden and facilitate diet adherence? What is the macronutrient distribution of the experimental diet? Diet instruction for the experimental diet would need to differ from the control as the protein powder contains 116 kcals.

All participants will follow a hypocaloric diet of 800 kcal below estimated energy requirements, energy needs is estimated by Mifflin Jeor St equation. Control group will follow a hypocaloric diet of 800 kcal below estimated energy requirements and intervention group will follow a hypocaloric diet of 916 kcal below estimated energy requirements, each of intervention group
subjects will receive 30-gram whey protein powders daily. The powders will be provided in a sachet form (each sachet containing 30-gram whey protein). Each 30 g of the whey protein supplement will be contained 116 kcal, 0.5 g of lipid, 0.4 g of carbohydrate, and 27.5 g of protein. Participants will be instructed to add one sachet to 250ml cold water and consume immediately, every evening. In control and intervention group the macronutrients percent will be carbohydrate 55%, fat 30% and protein 15%. Subjects will be asked to record powder intake and to check compliance, they will be followed by the dietitian via phone calls or SMS, every three days and, powders will be given to the subjects every 15 days. To create a variety in the diet while maintaining the general principles of diet, the same dietitian will give a dietary exchange list and a diet according to their food habits. In all visits (every 15 days), body weight will be measured with the accuracy of 100 gr using seca scale. A trained dietitian will design the energy requirements and macronutrient distribution and the same dietitian will be available to train participants on the diet. Due to financial limitation, no foods be provided to participants to ease participant burden and facilitate diet adherence.

- The use of accelerometers would better indicate physical activity over the 2-months as compared to a pre/post survey.

Due to financial limitation, the use of accelerometers is not possible. The IPAQ instrument has gained wide acceptance, and the short version in particular has been used in many international studies.

- What is meant by the exclusion criteria: no consumption of powders exceed 10% of total administered powders?

We corrected this sentence, at the end of the intervention, if the remained powders exceed 10% of total administered powders, that subject will be excluded from the study.

- Since the endocannabinoids are arachidonic acid metabolites, is arachidonic acid content of the diet controlled and similar between groups?

Although 2-AG and AEA are both agonists of CB1 receptors and they share the backbone of arachidonic acid in their structure they have different biosynthetic and degradation routes, which suggest they play a different modulatory role. Initially we wanted to measure serum arachidonic acid, but, due to financial limitation, we will measure the amount and type consumed fat by using Nut IV (the Hearst Corporation, San Bruno, CA).

- How will the Tanita calculate abdominal fat? Perhaps waist circumference should be taken as well.

We corrected this sentence, and waist circumference will be considered in the anthropometric measures. (Waist circumference will be assessed at above the iliac crest, just below the lowest rib margin at the end normal expiration to the nearest 0.5 cm. TANITA BC-418 body
composition analyzer will be used to calculate total body fat and fat percent, fat free mass and fat free mass percent).