PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	The Fiber-rich Foods to Treat Obesity and Prevent Colon Cancer trial study protocol: a randomized clinical trial of fiber-rich legumes targeting the gut microbiome, metabolome, and gut transit time of overweight and obese patients with a history of noncancerous adenomatous polyps
AUTHORS	Hartman, Terryl; Christie, Jennifer; Wilson, Annette; Ziegler, Thomas R.; Methe, Barbara; Flanders, W. Dana; Rolls, Barbara; Loye Eberhart, Blaine; Li, Jia; Huneault, Helaina; Cousineau, Ben; Perez, Miriam R.; O'Keefe, Stephen

VERSION 1 – REVIEW

REVIEWER	Wang, Fengzhong Chinese Academy of Agricultural Sciences
REVIEW RETURNED	19-Oct-2022

GENERAL COMMENTS	This is the first study to measure the effects of a high fiber diet on the human microbiota, metabolome, and colonic mucosal biomarkers of CRC over 12 months of intervention and to assess the effects of nutrition education on obesity and CRC risk at ~3 years.

REVIEWER	Orfila, Caroline University of Leeds
REVIEW RETURNED	31-Dec-2022

GENERAL COMMENTS	This manuscript describes the study protocol for a 12-month randomized control dietary intervention trial that investigates the effect of high-fibre legume consumption on weight status, plus a number of metabolic and clinical markers of disease. The title overstates the purpose, as the goal is not to treat obesity or prevent colon cancer, but to reduce body weight and reduce biomarkers associated with colon cell proliferation. Abstract: 'fiber-rich legumes, such as dry beans' - this sentence is problematic, because we don't generally eat dry beans (uncooked). On this point, research has shown that cooking beans affects their properties and fibre properties, this maybe worth exploring in the introduction, and also worth considering when providing cooked entrees to the participants (how will the legumes be cooked). The introduction may also mention the different types of fibre present in legumes including soluble, insoluble cell wall polysaccharides AND resistant starch. The abstract should mention the extended period. Introduction is adequate. There are two recent systematic reviews and meta-analyses on the effect of pulse consumption on markers of diabetes, these may be worth including.

	The methods need better description or links to papers that describe the methodologies in detail, including measures of insulin and insulin resistance, how the DEXA will be performed and where, what fit-bit information will be collected. Describe in more how blood, urine and stool will be collected and prepared. This information is crucial for reproducibility.

REVIEWER	Lozada-Hernandez, Edgar Efren Hospital Regional de Alta Especialidad del Bajio, Clinical Research
REVIEW RETURNED	05-Apr-2023

GENERAL COMMENTS	I sincerely appreciate the opportunity given to me to participate in the review of this protocol, I hope my observations will be useful to make it a better job. My observations are the following: I think the title mentions things that are not measured in the proposed methods, that is, they talk about cancer prevention and this is not possible to measure over time and with the proposed study methods and also the treatment of obesity, you mention that they expect a 1 kg weight reduction and refer to it as clinically significant, a question that is debatable because the reduction in that amount could be statistically significant but without clinical relevance, that is, the participant will continue to be obese or overweight after the study. I think that the title worded like this can lead to confusion, firstly because they are not proposing a methodologically adequate study to measure these two changes and secondly because they do not directly treat obesity, which is multifactorial, perhaps indirectly by reducing inflammation. , having changes in the microbiota and accelerating the transit, as they mention, could indirectly have this effect, but they cannot be sure, this may end up as a hypothesis in the discussion. I think that the wording of the title should be reconsidered and based on what will be measured and can be verified. The clinical and methodological justification for carrying out the study approach is clear to me, but sufficient and adequate references need to be written to provide a complete overview of the proposed intervention. The study is well supported in the background, however the frequency, route of administration and duration of the studied dose is not well supported in the development of the introduction, they only refer to a previous study by the same authors. The expected adverse events are not described and based on this to determine if the risk of undergoing this diet is acceptable, they only mention the risks and benefits of participating in the study but not of undergoing the diet. In the end, the writing ends by showing that this is intended to be a pilot study that will later be extrapolated to the general population and in greater numbers, so why not consider it that way, because you even mention that you may not find a statistically significant difference in some parameters. The sample size is not clear to me, they are based on the proliferation index measured with the Ki67, and they expect a difference between the two groups of 20%, they even mention that the difference could be 9.8%, with the numbers that you provide me I calculate a sample size of 176 for that power and with that difference could you please clarify what formula you used. What is the diagnostic performance of this test (Ki67) to detect the proliferation of the mucosa and that it is translated instead in the microbiome.

	Statistical analysis is proposed as the comparison between two groups, some to which the diet will be given and others to which it will not be worth doing an analysis before and after, that is, that the same patient is their control. Thanks again, waiting for your comments.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Fengzhong Wang, Chinese Academy of Agricultural Sciences:

1) **This is the first study to measure the effects of a high fiber diet on the human microbiota, metabolome, and colonic mucosal biomarkers of CRC over 12 months of intervention and to assess the effects of nutrition education on obesity and CRC risk at ~3 years.**
   Response: Thank you for your comment.

Reviewer: 2
Dr. Caroline Orfila, University of Leeds:

1) **This manuscript describes the study protocol for a 12-month randomized control dietary intervention trial that investigates the effect of high-fibre legume consumption on weight status, plus a number of metabolic and clinical markers of disease.**
   Response: Yes, thank you.

2) **The title overstates the purpose, as the goal is not to treat obesity or prevent colon cancer, but to reduce body weight and reduce biomarkers associated with colon cell proliferation.**
   Response: Thank your comment. We understand your concerns about our title; however, this is the official study title funded by the NIH listed under clinicaltrials.gov. If the editor wishes us to change the title, we could come up with something different. Thank you.

3) **Abstract: 'fiber-rich legumes, such as dry beans' - this sentence is problematic, because we don't generally eat dry beans (uncooked). On this point, research has shown that cooking beans affects their properties and fibre properties, this maybe worth exploring in the introduction, and also worth considering when providing cooked entrees to the participants (how will the legumes be cooked). The introduction may also mention the different types of fibre present in legumes including soluble, insoluble cell wall polysaccharides AND resistant starch.**
   Response: Thank you for your comment. We appreciate your feedback on our abstract and introduction. We added the word ‘cooked’ in front of dry beans in the abstract to clarify that we are using cooked legumes in our study. Additionally, we explain that we are using cooked legumes in our methods section. The manuscript word count limits us, and therefore, we did not go into detail about how cooking affects the properties of legumes; however, we added a sentence about the different types of fibre present in legumes and the benefits of soluble fibre to our introduction.

4) **The abstract should mention the extended period.**
   Response: Thank you. We agree that this is an interesting aspect of the study; however, it is not a main component, but exploratory, and the word count for the abstract limits us.

5) **Introduction is adequate. There are two recent systematic reviews and meta-analyses on the effect of pulse consumption on markers of diabetes, these may be worth including.**
Response: Thank you for bringing this to our attention. We have added one of the reviews (see reference below) to the introduction of our manuscript.

Hafiz MS, Campbell MD, O'Mahoney LL, Holmes M, Orfila C, Boesch C. **Pulse consumption improves indices of glycemic control in adults with and without type 2 diabetes: a systematic review and meta-analysis of acute and long-term randomized controlled trials.** Eur J Nutr. 2022 Mar;61(2):809-824.

6) **The methods need better description or links to papers that describe the methodologies in detail, including measures of insulin and insulin resistance, how the DEXA will be performed and where, what fit-bit information will be collected.**
Response: Thank you for asking us to clarify this. The Fitbit Aria is a smart scale (https://www.fitbit.com/global/us/products/scales/aria-air) that is used for weekly home self-monitoring and is not an outcome measure. This is indicated under the 'Anthropometry' section. The word count limits our ability to go into detail on the methodologies used to perform DEXA scans and measure insulin and insulin resistance. Therefore, we added a sentence in the 'Data Collection' section stating that this information is provided in our Supplementary Materials and included this additional document with our revised submission.

7) **Describe in more how blood, urine, and stool will be collected and prepared. This information is crucial for reproducibility.**
Response: Thank you for asking us to clarify this as well. The word count limits our ability to go into detail about these methods. Therefore, we have added a section to our Supplementary Materials to explain these methods in further detail (including additional references) and added a sentence indicating this in the 'Data Collection' section.

Reviewer: 3
Dr. Edgar Efren Lozada-Hernandez, Hospital Regional de Alta Especialidad del Bajio:

1) **I think the title mentions things that are not measured in the proposed methods, that is, they talk about cancer prevention and this is not possible to measure over time and with the proposed study methods and also the treatment of obesity, you mention that they expect a 1 kg weight reduction and refer to it as clinically significant, a question that is debatable because the reduction in that amount could be statistically significant but without clinical relevance, that is, the participant will continue to be obese or overweight after the study. I think that the title worded like this can lead to confusion, firstly because they are not proposing a methodologically adequate study to measure these two changes and secondly because they do not directly treat obesity, which is multifactorial, perhaps indirectly by reducing inflammation. , having changes in the microbiota and accelerating the transit, as they mention, could indirectly have this effect, but they cannot be sure, this may end up as a hypothesis in the discussion. I think that the wording of the title should be reconsidered and based on what will be measured and can be verified.**
Response: Thank you for your comments. We understand your concerns about our title however, this is the official study title funded by the NIH listed under clinicaltrials.gov. If the editor wishes us to change the title, we could come up with something different. We also appreciate your comment regarding the clinical significance of 1 kg weight loss. According to Blackburn., even small amounts of weight loss have been shown to improve disorders associated with obesity, such as hypertension and hyperlipidemia[1]. We have added this reference to the sentence about the clinical significance of 1 kg weight change under our

'Statistical considerations' section. If necessary, we could remove the word 'clinical' from this sentence. Additionally, through 4/15/2023 a total of 19 research participants passed the 6-month follow-up time point (primary end point). Mean weight loss at 6 months for the group as a whole (both diet treatments) was -9.2 (SD 8.0) pounds overall, and -9.5 (SD 8.0) and -8.8 (SD 8.4) pounds among men and women, respectively. Therefore, we are seeing well over 1 kg of weight loss at 6 months. Thank you.

2) **The clinical and methodological justification for carrying out the study approach is clear to me, but sufficient and adequate references need to be written to provide a complete overview of the proposed intervention. The study is well supported in the background, however the frequency, route of administration and duration of the studied dose is not well supported in the development of the introduction, they only refer to a previous study by the same authors. The expected adverse events are not described and based on this to determine if the risk of undergoing this diet is acceptable, they only mention the risks and benefits of participating in the study but not of undergoing the diet. In the end, the writing ends by showing that this is intended to be a pilot study that will later be extrapolated to the general population and in greater numbers, so why not consider it that way, because you even mention that you may not find a statistically significant difference in some parameters.**
Response: - Thank you for your comment. We are limited by the word count of our manuscript; however, we referenced two previously conducted studies that provided fiber at similar doses and included more detail about the intervention in our Methods section. We did not mean to include the word 'pilot' in our Extended Follow-up section. Thank you for catching this. We have removed the word pilot from this section. We do not consider this a pilot study since we already conducted a previous pilot study. This study is a randomized well controlled trial. Also, thank you for pointing out that we did not include the expected adverse events from the diet intervention. We have added a sentence on this under the 'Potential Risk and Benefits to Participants' section.

3) **The sample size is not clear to me, they are based on the proliferation index measured with the Ki67, and they expect a difference between the two groups of 20%, they even mention that the difference could be 9.8%, with the numbers that you provide me I calculate a sample size of 176 for that power and with that difference could you please clarify what formula you used. What is the diagnostic performance of this test (Ki67) to detect the proliferation of the mucosa and that it is translated instead in the microbiome.**
Response: Thank you for your comment. The key assumptions for the power calculations were that the standard deviation of the difference was 4 and that mean in 1 group would differ from that in the other group by about 3.4. For a mean in one group of 35.1 (about what O'Keefe saw in some populations), 9.8% lower translates to a difference in means (absolute scale) of 3.43 – as we used in calculations. With these assumptions, power was calculated in SAS proc power. For 80% power, a sample size of at least 23 per group would suffice.

```
proc power;
    twosamplemeans test=diff  meandiff = 3.4  stddev = 4
    npergroup = .  power = .80;
run;
```

4) **Statistical analysis is proposed as the comparison between two groups, some to which the diet will be given and others to which it will not be worth doing an analysis before and after, that is, that the same patient is their control.**

Response: Thank you for your comment. Each patient is being considered as their own control in our multivariate analysis. As one of the early steps in of our analysis we will consider before and after within group differences. Primary analyses will initially use the net change in weight at 6 months, and contrast the change at six months in the intervention with that in the controls group (difference of differences).

Reference:

1. Blackburn G. Effect of degree of weight loss on health benefits. *Obes Res*. 1995;3 Suppl 2:211s-6s.

VERSION 2 – REVIEW

REVIEWER	Lozada-Hernandez, Edgar Efren Hospital Regional de Alta Especialidad del Bajio, Clinical Research
REVIEW RETURNED	16-May-2023

GENERAL COMMENTS	Thank you for the response to my observations, which I insist only have the objective of doing a better job. The main objectives of the work are two according to its wording: to treat obesity and secondarily to evaluate the effect of weight loss in reducing the risk of cancer, with a diet rich in fiber and up to that point it is correct. However the conversion of the adenomatous polyp to cancer depends a lot on its characteristics, you only mention 1>- polyp > 0.5 cm, depending on the author but a 1.5 cm tubular polyp has a risk of malignancy of 2% and if it is villous and measures 3 cm it is 35%. I think that the characteristics of the polyp should be taken into account in particular and not leave them so general, because perhaps the occurrence is not due to the diet but to the characteristics of the polyp and suddenly expect that the pure roasting will solve this not because the characteristics of the simple polyp determine that the risk of conversion is 2% but if it is a hairy adenoma larger than 3 cm the risk is 35% I do not believe that the decrease of one kg of weight or 2 as you refer in your description will prevent cancer, the sample size is still calculated with a different measure to the main objective which is weight loss.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 3
Dr. Edgar Efren Lozada-Hernandez, Hospital Regional de Alta Especialidad del Bajio
Comments to the Author:
Thank you for the response to my observations, which I insist only have the objective of doing a better job.
The main objectives of the work are two, according to its wording: to treat obesity and secondarily to evaluate the effect of weight loss in reducing the risk of cancer, with a diet rich in fiber, and up to that point it is correct.

However, the conversion of the adenomatous polyp to cancer depends a lot on its characteristics, you only mention 1>- polyp > 0.5 cm, depending on the author, but a 1.5 cm tubular polyp has a risk of malignancy of 2% and if it is villous and measures 3 cm it is 35%. I think that the characteristics of the polyp should be taken into account in particular and not leave them so general, because perhaps the occurrence is not due to the diet but to the characteristics of the polyp and suddenly expect that the pure roasting will solve this not because the characteristics of the simple polyp determine that the risk of conversion is 2% but if it is a hairy adenoma larger than 3 cm the risk is 35% I do not believe that the decrease of one kg of weight or 2 as you refer in your description will prevent cancer, the sample size is still calculated with a different measure to the main objective which is weight loss.

Thank you for your comment. We would like to clarify that our study primarily focuses on assessing cancer prevention rather than cancer treatment. One of our study objectives is to evaluate the ability of a high-legume diet versus a control diet to reduce body weight. We also aim to characterize changes in biomarkers related to insulin resistance and systemic inflammation, profile the fecal microbiome and metabolome, assess gut transit time, and examine mucosal biomarkers associated with colorectal cancer risk. Our research focuses on modifiable environmental factors, specifically dietary choices, that influence cancer risk rather than genetic abnormalities. Since our study is a cancer prevention study that targets healthy individuals, future results may not be generalizable to cancer patients. To address this limitation, we have added a sentence to our revised manuscript in the “Strengths and Limitations” section on page 3.

Additionally, thank you for your comment regarding the sample size. We calculated sample size and power for both weight loss and detecting a difference in mucosal proliferation between groups over 6 and 12 months. We have updated our Statistical Considerations section on power at the bottom of page 10 to clarify this. For weight loss, power is based on a difference in the trajectory of weight loss between groups over 6- and 12- mos. A clinically significant difference in weight loss is assumed to be 1.0 kg $\pm$ 0.9 kg. We expect to observe a larger difference at 6 mos. (e.g., 1.5 kg) and a 1.0 kg difference maintained at 12 mos. With a final sample of 60 participants, we would have >95% power for a weight loss difference of 1.0 kg or even 0.8 kg at both time points. If weight change in men differs from women by 0.8 or 0.9 kg ($\pm$ 1.27 kg), the power to detect the difference is 67% or 77%, respectively. These numbers are more than sufficient to observe clinically meaningful changes in secondary outcomes. They will also facilitate our global microbiome and metabolic analyses where exact power calculations are not feasible. Lastly, in our limitations section on page 3, we indicated that the study is not sufficiently powered to evaluate if the anticipated changes in mucosal biomarkers predict polyp recurrence. However, we estimated this in our Statistical Considerations section.

VERSION 3 – REVIEW

REVIEWER	Lozada-Hernandez, Edgar Efren Hospital Regional de Alta Especialidad del Bajio, Clinical Research
REVIEW RETURNED	27-Nov-2023

GENERAL COMMENTS	It has been insisted that the methodology to evaluate their outcome variables is not adequate, the proposition that reducing weight will reduce the risk of cancer in this type of patient is very difficult to establish first because the malignant transformation of a polyp depends of at least the following characteristics (which are not analyzed in the inclusion criteria since they only mention more

	than one polyp and that it is greater than 0.5 cm, thinking that randomization will resolve the bias): 1. **Size of the Polyp**: The larger the polyp, the greater the risk of malignancy. Polyps larger than 2 cm have a more than 40% chance of being malignant. 2. **Dysplasia Severity**: The presence and severity of dysplasia (abnormal cells) in the polyp also influence the risk. Polyps with severe dysplasia have a higher likelihood of being malignant. 3. **Type of Adenoma**: Certain types of adenomas, such as tubulovillous or villous adenomas, have a higher risk of malignant transformation compared to others like tubular adenomas. 4. **Number of Polyps**: Multiple adenomatous polyps increase the risk of one or more of them becoming malignant. 5. **Patient Factors**: Patient-related factors such as genetics, family history of colorectal cancer, and personal history of polyps or colorectal cancer can also impact the risk. 6. It's important to note that while adenomatous polyps are a significant risk factor for colorectal cancer, not all adenomas become malignant. Regular screening and removal of these polyps can significantly reduce the risk of colorectal cancer and it is not mentioned how those polyps were treated. The second and most important thing is to insist that the loss of 1 kg of weight is clinically significant, because from there derives the sample size that will be used and, secondarily, the differences between the groups.

VERSION 3 – AUTHOR RESPONSE

Reviewer: 1

Dr. Edgar Efren Lozada-Hernandez, Hospital Regional de Alta Especialidad del Bajio

Comments to the Author:

It has been insisted that the methodology to evaluate their outcome variables is not adequate, the proposition that reducing weight will reduce the risk of cancer in this type of patient is very difficult to establish first because the malignant transformation of a polyp depends of at least the following characteristics (which are not analyzed in the inclusion criteria since they only mention more than one polyp and that it is greater than 0.5 cm, thinking that randomization will resolve the bias):

1. **Size of the Polyp: The larger the polyp, the greater the risk of malignancy. Polyps larger than 2 cm have a more than 40% chance of being malignant.**

2. **Dysplasia Severity: The presence and severity of dysplasia (abnormal cells) in the polyp also influence the risk. Polyps with severe dysplasia have a higher likelihood of being malignant.**

3. **Type of Adenoma: Certain types of adenomas, such as tubulovillous or villous adenomas, have a higher risk of malignant transformation compared to others like tubular adenomas.**

4. **Number of Polyps: Multiple adenomatous polyps increase the risk of one or more of them becoming malignant.**

5. **Patient Factors: Patient-related factors such as genetics, family history of colorectal cancer, and personal history of polyps or colorectal cancer can also impact the risk.**

Thank you for your insightful comments. We acknowledge the importance of the factors you've highlighted in assessing the risk of malignancy in adenomatous polyps. We agree that these factors are critical in a comprehensive understanding of colorectal cancer risk. Our study primarily aims to explore the effect of a high-fiber diet on weight management and cancer risk reduction in patients with a history of adenomatous polyps. While we acknowledge the importance of polyp size, dysplasia severity, adenoma type, number of polyps, and patient-specific factors in determining malignancy risk, these were beyond our study's scope. Our study was not sufficiently powered to assess the malignant transformation of polyps. We can expect significant changes in mucosal biomarkers within the intervention period, but we did not intend to follow participants to recurrence endpoints as it takes years for cancer to develop.

Additionally, our inclusion criteria and randomization were designed to minimize biases and focus on the study's objectives within its scope. While we included participants based on the presence of adenomatous polyps in general (colonoscopy within three years that found/removed ≥1 adenoma >0.5 cm), we did not consider additional polyp characteristics due to the lack of power to adequately assess the malignant transformation of polyps. Furthermore, weight loss is associated with improved insulin sensitivity, blood lipid profiles, and reduced colorectal inflammation, thereby decreasing CRC risk[1,2].

Overall, our study centers on the systemic effects of diet on weight and biomarkers associated with colorectal cancer risk rather than detailed polyp analysis. While factors such as polyp size, dysplasia severity, adenoma type, and number of polyps are crucial in assessing cancer risk, these factors are beyond the purview of our current research focus. We recognize their importance and have expanded a sentence in our limitations section to address this.

Revision (Page 3; Strengths and limitations of the study): **“Dietary compliance in the intervention group is a potential limitation, and the study is not sufficiently powered to evaluate if the anticipated changes in mucosal biomarkers predict polyp recurrence or malignant transformation.”**

6. It's important to note that while adenomatous polyps are a significant risk factor for colorectal cancer, not all adenomas become malignant. Regular screening and removal of these polyps can significantly reduce the risk of colorectal cancer, and it is not mentioned how those polyps were treated.

Thank you for your comment. We have clarified that a history of polyps with removal was part of our eligibility criteria.

Revision (Page 5; Eligibility criteria): Inclusion: (1) Free-living adults 40-75 yrs. old, (2) BMI 25-40 kg/m^2, (3) **colonoscopy within three years that found/removed ≥1 adenoma >0.5 cm,** (4) English speaking, (5) ambulatory, (6) able to provide informed consent.

The second and most important thing is to insist that the loss of 1 kg of weight is clinically significant, because from there derives the sample size that will be used and, secondarily, the differences between the groups.

Thank you for your comment. According to Blackburn, a 5% decrease in weight is clinically significant due to improvements in blood pressure, lipid profile, and insulin sensitivity[3]. We have updated the sentence regarding the clinically significant change in weight in our 'Statistical considerations' section to address that a significant difference in weight loss between the two groups is considered to be 1.0 kg + 0.9 kg.

Revision (Page 10: Statistical considerations): **"A 5% decrease in weight is clinically significant due to improvements in blood pressure, lipid profile, and insulin sensitivity (65). A significant difference in weight loss between the two groups is considered to be 1.0 kg ± 0.9 kg."**

References:

1. Pendyala S, Neff LM, Suárez-Fariñas M, Holt PR. Diet-induced weight loss reduces colorectal inflammation: implications for colorectal carcinogenesis. *Am J Clin Nutr*. Feb 2011;93(2):234-42. doi:10.3945/ajcn.110.002683
2. Beeken RJ, Croker H, Heinrich M, et al. The Impact of Diet-Induced Weight Loss on Biomarkers for Colorectal Cancer: An Exploratory Study (INTERCEPT). *Obesity (Silver Spring)*. Nov 2017;25 Suppl 2(Suppl 2):S95-s101. doi:10.1002/oby.21984
3. Blackburn G. Effect of degree of weight loss on health benefits. *Obes Res*. Sep 1995;3 Suppl 2:211s-216s. doi:10.1002/j.1550-8528.1995.tb00466.x

VERSION 4 – REVIEW

REVIEWER	Lozada-Hernandez, Edgar Efren Hospital Regional de Alta Especialidad del Bajio, Clinical Research
REVIEW RETURNED	02-Jan-2024

GENERAL COMMENTS	I appreciate the opportunity to evaluate the protocol, the observations and concerns about the scientific relevance of the study have been completed, I hope that the results are as expected and can contribute to the prevention of this neoplasia.