August 16th 2024

Re: Response to reviewers for "The Portfolio Diet and HbA1c in adults living with type 2 diabetes mellitus: a patient-level pooled analysis of two randomized dietary trials" Manuscript ID: nutrients-3142697

We thank the reviewer for their insightful comments. We have addressed each of the comments point-by-point below and feel the revisions have improved this manuscript. Our responses to each of the comments are indicated below under the response heading in blue text.

Reviewer 2

Comment 1: This manuscript attempts to explore the relationship between adherence to the Portfolio Diet and the reduction of HbA1c levels in adults living with type 2 diabetes mellitus (T2DM) by pooling patient-level data from two randomized dietary trials. While the research topic is relevant to diabetes management, the manuscript falls short in several key areas that must be addressed to meet the standards expected in this field.

Response 1: We thank the reviewer for their thoughtful comments. We are glad to hear our work is on a topic that they consider relevant to diabetes management. We agree the manuscript could be improved and have addressed each of the comments point-by-point below.

1. Introduction

Comment 2: The manuscript lacks a clear articulation of the research hypothesis and objectives. The authors mention the aim but fail to provide a concise statement of the hypothesis being tested. This omission significantly undermines the focus and readability of the paper. The authors must explicitly state the hypothesis and clarify the objectives to ensure that the study's direction is evident from the outset.

Response 2: Thank you. We agree and have included a hypothesis and an objective in the introduction section. The updated section now reads:

> *"We therefore investigated the effect of the Portfolio Diet in T2DM by analysing patient-level data from two completed randomized controlled trials of low GI diets compared to high cereal fibre diets in adults living with T2DM, Diabetes Management with a low Glycemic Index diet (DMGI) and DM-Magnet Residence Imagining (DM-MRI). As the Portfolio Diet is made up of many low-GI foods, it was hypothesized that the Portfolio Diet would be inversely associated with HbA1c in adult participants living with T2DM. The objective of the present study was to assess the association between adherence to the Portfolio Diet and change in HbA1c in an exploratory analysis of 2 completed trials on low-GI diets in T2DM."*

2. Methods

Comment 3: Given the centrality of the Portfolio Diet to this study, the current level of detail provided about this diet in the main text is insufficient. While references are cited, the manuscript itself must contain a thorough and self-sufficient explanation of the Portfolio Diet. Relying on external references is inadequate and diminishes the manuscript's comprehensiveness.

Response 3: Thank you for the wise suggestion. We agree and have added a section within the methods to describe the Portfolio Diet. Please see the new section below:

*"**The Portfolio Diet***

The Portfolio Diet is a "portfolio" of key foods with established cholesterol-lowering properties (15). The diet includes: 45 grams of nuts and seeds (almonds, walnuts, pumpkin seeds, peanut butter, etc.), 20 g of viscous fibre (from psyllium, oats, oatbran, barley, eggplant, okra, apples, oranges, etc.), 50 g plant protein (from tofu, soymilk, plant-based meat alternatives, beans, chickpeas, lentils, etc.), 2 g plant sterols (from fortified foods and supplements), and 45 g plant-derived MUFA (from extra virgin olive oil, cold pressed canola and soybean oil, avocado fruit, etc.) (20). These gram targets are for a 2,000kcal diet. In addition to these 5 categories, the Portfolio Diet also has a background diet of <7% of total energy from saturated fat and <200mg/day of cholesterol, in alignment with the National Cholesterol Education Program (NCEP) II diet (33)."

Comment 4: Moreover, the lack of sensitivity analyses is a glaring oversight. Without these analyses, the robustness of the findings remains questionable. The authors must conduct and present sensitivity analyses to strengthen the validity of their results.

Response 4: Thank you, we agree and have performed a two separate sensitivity analyses, one with dietary GI and one with change in body weight as possible mediators.

When dietary GI was included as a covariate in the multiple linear regression model of c-PDS and HbA1c, the association was attenuated; the beta coefficient was halved and the modeled association was no longer significant (β: -0.04% per c-PDS point, 95% CI:-0.07, -0.02, *P*= 0.001 versus β: -0.02% per c-PDS point, 95% CI:-0.05, 0.01, *P*= 0.287). Therefore, as predicted, this sensitivity analysis suggests dietary GI may be a key mediator and mechanism for how the Portfolio Diet may improve HbA1c.

When change in body weight change was included as a covariate in the multiple linear regression model of c-PDS and HbA1c, the association was attenuated by 25%, however, the association remained significant (β: -0.04% per c-PDS point, 95% CI:-0.07, -0.02, *P*= 0.001 versus β: -0.03% per c-PDS point, 95% CI:-0.05, -0.01, *P*= 0.005). Therefore, a reduction in body weight may explain some of the associated benefits of the Portfolio Diet on HbA1c but additional mechanisms drive this association.

Both these results have been added to the supplement (**Table S4**) and we have discussed the role of GI and body weight as potential mechanisms explaining the association between the Portfolio Diet and glycemic control.

3. Results

Comment 5: The manuscript reports that adherence to the Portfolio Diet was generally low, yet it proceeds to draw conclusions about the diet's efficacy. This is a fundamental flaw in the study design and interpretation. The low adherence level raises serious doubts about whether the findings can truly reflect the diet's effect. This issue is the most significant flaw in the paper and calls into question the study's overall validity. Furthermore, the authors have neglected to analyze the characteristics of participants who did not adhere to the diet. This omission prevents a full understanding of how non-adherence may have skewed the results. A detailed analysis of these participants is essential and must be included.

Response 5: We thank the reviewer for their comment.

This is a pooled analysis from two separate trials, to use as a cohort of participants consuming a range of Portfolio Diet foods. While adherence was low in the pooled data of all participants (control and test

groups), the range of adherence at 6 months was broad, ranging from 0 to 74%. As this was a secondary analysis of two randomized dietary trials (low glycemic index intervention compared to high cereal fibre control diet), we did not expect the pooled adherence to the Portfolio Diet to be high and have included a section within the discussion to clarify these points. Please see the revised section below:

> *"Adherence to the Portfolio Diet increased from 16% to 18% at 6-months. While the pooled adherence was low there was a large range of adherence across the participants, ranging from 0 to 60% and 0 to 74% at baseline and 6 months respectively. Furthermore, when separated by original trial intervention, the 6-month c-PDS for those in the control groups was 2.6 points (10%), ranging from 0 to 13 points (0% to 52%) and for those in the test groups was 5.8 points (23%), ranging from 0.5 to 18.3 points (2% to 74%). Also, as Figure S2 presents, adherence generally decreased in the control (high fibre dietary advice) and increased in the test (low GI dietary advice) group, as expected. The mean change in c-PDS from baseline to 6-months for those in the control groups was -1.5 points, ranging from -7.8 to 7.4 points. The mean change for those in the test groups was 1.7 points, ranging from -6.3 to 9.4 points."*

Using the data generated from this study, we were able to estimate the level of adherence to the Portfolio Diet needed for a clinically meaningful improvement in HbA1c (0.3%, as per EMA and FDA guidance) to be a 7.5-point increase (out of 25-points) in the c-PDS, corresponding to 30% adherence to the Portfolio Diet. A future trial demonstrating the direct effect of the Portfolio Diet will allow us to determine the effectiveness of the Portfolio Diet on diabetes management.

4. Discussion

Comment 6: The discussion on the association between the Portfolio Diet and HbA1c reduction is superficial at best. The manuscript would benefit from a much deeper exploration of the biological mechanisms that might explain these effects. The current discussion lacks depth and fails to provide the necessary insights into the study's findings.

Response 6: *Thank you, we agree and have written a section to provide a deep exploration of the biological mechanisms to potentially explain the association between the Portfolio Diet and glycemic control, including substitution of high with low GI foods, satiety, delayed gastric emptying, change in body weight, production of short chain fatty acids and inflammation. Please see our newly added paragraphs in the discussion.*

> *"When considering potential mechanisms through which the Portfolio Diet may work to improve HbA1c, the displacement of high GI carbohydrates foods with low GI foods such as nuts, beans, lentils, etc., has been previously observed to improve cardiometabolic outcomes(38, 39). In addition to dietary GI, viscous fibre is believed to delay gastric emptying, increasing satiety and decreasing energy intake(40). As half of the participant (48%) included in our analysis were considered obese, the potential role of dietary interventions on satiety, which may influence adipose tissue distribution and improve glycemic control, is an important mechanism to consider. In the DM-MRI study change in HbA1c was associated with change in body weight (32). In our mediation analysis, we observed that while body weight attenuated the association between the Portfolio Diet and HbA1c by 25%, the association remained significant (**Table S4**). This finding suggests that while a reduction in body weight may explain some of the associated benefits of the Portfolio Diet on HbA1c, additional mechanisms drive this association.*
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> *Additionally, short-chain fatty acids (SCFA), produced by the fermentation of fibre-rich foods by bacteria in the colon, may inhibit fatty acid lipolysis, reducing levels of circulating free fatty acids,*

and may improve tissue insulin sensitivity (41, 42). Inflammation may also be an important mechanism, as the Portfolio Diet has previously been observed to improve CRP (16). These observed anti-inflammatory effects of the diet may be related to an increase in dietary polyphenols from nuts and dietary pulses (45, 46) which may have downstream influences on chronic diseases (43, 44). Moreso, the replacement of carbohydrates with MUFA has also been observed to improve markers of inflammation (47).”

Comment 7: Additionally, the discussion of confounding factors is inadequate. The authors must address any residual confounding factors that could still be influencing the results. Ignoring these potential influences weakens the credibility of the study's conclusions.

Response 7: We thank the reviewer for pointing this out. We agree and have included a statement in the limitations section of the manuscript. The section reads:

“Residual confounding is an additional limitation; however, we did adjust our multilinear regression analyses for several potential confounding variables. We also attempted to explore factors which may influence the results, including mediation analyses for dietary GI and for change in body weight, both of which attenuated the association. However, the possibility of residual confounding, including unknown factors, cannot be ruled out.”