PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	A systematic review of nutrigenetics, omega-3 and plasma lipids/lipoproteins/apolipoproteins with evidence evaluation using the GRADE approach
AUTHORS	Keathley, Justine; Garneau, Véronique; Marcil, Valérie; Mutch, David; Robitaille, Julie; Rudkowska, Iwona; Sofian, Gabriela; Desroches, Sophie; Vohl, Marie-Claude

VERSION 1 – REVIEW

REVIEWER	Milenkovic, DraganMilenkovic, Dragan Universite Clermont Auvergne, Nutrition Humaine
REVIEW RETURNED	31-Aug-2021

GENERAL COMMENTS	In the manuscript submitted by Horne et al., the authors performed systematic review analysis of existing data on gene-nutrient interactions by evaluating associations between genetic polymorphism and omega-3 fatty acid intake on plasma lipid, lipo- and apolipoprotein responsiveness. The manuscript is clear, well written and performed using defined guidelines. There are few points that the authors could address: - as there are few studies in the topic, an additional paragraph on nutrigenetics and other nutrients, like phytosterols or polyphenols for example, could be added to demonstrate the role of genetic polymorphism in nutrition - the same could be also for importance of sex in responsiveness to food intake, there are few examples for other nutrients like polyphenols - the authors concluded that there are few studies performed, could authors provide several ideas for future work?

REVIEWER	Rozga, Mary Academy of Nutrition and Dietetics
REVIEW RETURNED	31-Aug-2021

GENERAL COMMENTS	This is an important and timely topic area. I appreciate the authors' utilization of the GRADE method. However, there is a huge methods issue in this paper, and it should not be published as is. The entire point of a systematic review is to gather and analyze all studies that answer the research question and meet inclusion criteria. Selecting studies to grade from the included studies based on study findings completely invalidates the systematic review. If this manuscript moves on to the next review phase, the separate research questions should be specified with specific inclusion criteria for that question. Then ALL studies should be included when grading evidence.

	Abstract • In objectives, suggest replacing “(level)” with “(confidence)”. • Design: The intervention/exposure isn’t clear. Interventions that utilize information on plasma-lipid related gene info to guide the intervention? Studies that examine the association between these genes and outcomes? I might be helpful to list the included study designs and/or to list these as two separate research questions. • Design: Please specify the population. • Design: As it is phrased, it looks like evidence was only included if it had a certain result, which is not appropriate for systematic reviews. All studies answering the a priori research question should be included and evaluated. • Design: List databases searched and inclusion dates of articles. • Results: All studies meeting inclusion criteria should be included. Introduction • Objective: This should be stated in PICO format. What is the population. What specifically is the intervention or exposure? There are several questions being asked and answered and these should all be listed separately. Only use the word “effect” when referring to trials and “relationship” or “association” can be used for observational studies. Methods • What was the start date of the search? • This helps clarify the intervention/exposure a little more, but it would be helpful to actually list out the separate PICO questions someplace. It seems more like a scoping review as is. • A table that describes the inclusion/exclusion for each type of study/research question you are investigating would be helpful. There are a few different research questions that are being lumped together. • Evidence grading: This section is very confusing. There should never be studies that are “selected for grading” after studies have been included. All included studies are included in grading. Selecting studies after inclusion defeats the entire purpose of a systematic review. It makes no sense to choose the significant studies and then grade them. It is true that having two studies can result in high quality evidence, but that’s if those are the only studies answering the same question, they are RCTs, have large sample sizes, are consistent, etc. It is not ok to choose specific studies from included studies for grading. What if there were four studies that answered the question, two showed no relationship and two showed a relationship. We can’t simply ditch the two that found no relationship and say the relationship is positive. Maybe I am missing what the authors actually did here, in which case the process needs to be clarified. • It is completely against valid systematic review methods to “filter out evidence that would be deemed low or very low quality”. That is not a systematic review.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Dragan Milenkovic, Universite Clermont Auvergne

Comments to the Author:

In the manuscript submitted by Horne et al., the authors performed systematic review analysis of existing data on gene-nutrient interactions by evaluating associations between genetic polymorphism and omega-3 fatty acid intake on plasma lipid, lipo- and apolipoprotein responsiveness. The manuscript is clear, well written and performed using defined guidelines. There are few points that the authors could address:

COMMENT

- as there are few studies in the topic, an additional paragraph on nutrigenetics and other nutrients, like phytosterols or polyphenols for example, could be added to demonstrate the role of genetic polymorphism in nutrition

RESPONSE

Dear Dr. Milenkovic: Thank you for taking the time to review our manuscript and provide several helpful comments aimed to help strengthen the manuscript. We have worked these into our manuscript, while maintaining respect for the author guidelines including word limitations. In response to your first comment, we have added to the first paragraph in the introduction (page 3, lines 73-75) by outlining two specific examples of nutrigenetic associations.

COMMENT

- the same could be also for importance of sex in responsiveness to food intake, there are few examples for other nutrients like polyphenols

RESPONSE

We have added to this part of the discussion, providing an example of sex-specific responsiveness to resveratrol (page 21, lines 317-319)

COMMENT

- the authors concluded that there are few studies performed, could authors provide several ideas for future work?

RESPONSE

Note that there are actually many studies that have been conducted (n=65) but we instead conclude that the level of evidence for most of the identified gene-diet associations is weak, in part due to lack of replication. We further provide several directions for future research throughout the discussion: we encourage researchers to further explore the use of nutri-GRSs, include sex-specific analyses, use unbiased or non-hypothesis driven approaches to derive nutri-GRSs, and replicate significant results from studies that have yet to be replicated (e.g. Chen et al. 2019). If there's anything more specific that you recommend we include, kindly let us know and we would be happy to consider working it into our discussion.

Reviewer: 2
Dr. Mary Rozga, Academy of Nutrition and Dietetics

COMMENT

Comments to the Author:
This is an important and timely topic area. I appreciate the authors' utilization of the GRADE method. However, there is a huge methods issue in this paper, and it should not be published as is. The entire point of a systematic review is to gather and analyze all studies that answer the research question and meet inclusion criteria. Selecting studies to grade from the included studies based on study findings completely invalidates the systematic review. If this manuscript moves on to the next review phase, the separate research questions should be specified with specific inclusion criteria for that question. Then ALL studies should be included when grading evidence.

RESPONSE

Dear Dr. Rozga: Thank you kindly for taking the time to review our manuscript and provide several comments aimed to help strengthen this work. Indeed, there appears to be a misunderstanding related to our methods so we would particularly like to thank you for pointing out this confusion and allowing us to clarify. We agree with you that a critical flaw of a systematic review would be to cherry pick studies with significant findings for a particular intervention and outcome and exclude those with non-significant findings for the same intervention and outcome. However, this is not what we have done. We have clarified our methods briefly for you in the paragraphs below, and revised our manuscript as outlined in our responses to your comments that follow. We did not “select studies to grade from the included studies based on study findings” and agree that this would invalidate the systematic review.

First, we had to broadly and systematically searched for any study that evaluated omega-3 dietary/supplemental interventions or exposures on plasma HDL-c, LDL-c, total-c, LDL particle size, TG or apolipoproteins (or their ratios) and that assessed the influence of genetic variation on the plasma response (objective 1). We started by conducting this broad search given that the precise genetic variants influencing plasma lipid/apolipoprotein responsiveness to omega-3 had not yet been identified through a systematic review. We then aimed to identify which genetic variants, if any, have strong evidence to influence this response (objective 2).

Upon identifying all studies on this topic, we then narrowed down which gene-diet-lipid/apolipoprotein outcomes would be selected for evidence grading. We had to narrow down the nutrigenetic associations/interactions included in evidence grading because hundreds of combinations of genetic variants and plasma lipid/apolipoprotein outcomes were identified in our initial search. Given that the GRADE approach suggests that non-replicated results do not generally result in strong evidence, these non-replicated nutrigenetic associations/interactions were excluded from evidence grading. For example, we did not conduct evidence grading for ZNT8, rs13266634, omega-3 intake and plasma HDL-c or TG (assessed by Hosseini-Esfahani et al. 2017) given that only one study assessed this.

We therefore only included nutrigenetic associations/interactions that had been replicated. However, for the replicated nutrigenetic associations/interactions included in evidence grading, we still included studies with non-significant findings. For example, while the association between PPARg2 rs1801282, omega-3 and LDL-c had been replicated this nutrigenetic association was included in evidence grading. When considering the body of evidence, which included several studies showing non-significant results (more details in Table 4), we concluded that “Strong evidence suggests that genetic variation in PPARg2 (rs1801282) does not influence LDL-c responses to omega-3s (EPA+DHA).” This is just one example where the results were conflicting, with some studies included in evidence grading having non-significant results and others having significant results.

Using the GRADE approach to compare studies that tested different genetic variants would of course be like comparing apples to oranges and would be a critical flaw, and I don’t think this is what you are suggesting. Instead, we compared apples to apples by stratifying nutrigenetic

associations/interactions that had been replicated, but we still included studies on these same nutrigenetic associations/interactions that had non-significant findings when we completed evidence grading.

So we did not exclude all studies with non-significant findings from the GRADE approach; we stratified the nutrigenetic topics based on the genetic variants and outcomes evaluated, and then prioritized nutrigenetic associations/interactions for evidence grading on the basis of replication. ALL studies that assessed the same genetic variant(s) and outcomes were then included in evidence grading, regardless of if the results were significant or not.

We hope this helps to clarify our methods, and should you still have questions or concerns we would be happy to address these. Further details are provided in our responses below, and changes have been tracked in our manuscript.

COMMENT

Abstract
• In objectives, suggest replacing “(level)” with “(confidence)”.

RESPONSE

Replaced.

COMMENT

• Design: The intervention/exposure isn’t clear. Interventions that utilize information on plasma-lipid related gene info to guide the intervention? Studies that examine the association between these genes and outcomes? I might be helpful to list the included study designs and/or to list these as two separate research questions.

RESPONSE

Revised to “Included studies for the narrative synthesis assessed nutrigenetic associations/interactions for genetic variants influencing the plasma lipid, lipo- and/or apolipoprotein response to omega-3 fatty acid intake.”… “Specific nutrigenetic associations/interactions were then prioritized for evidence grading if they had been replicated, while still including studies evaluating the same nutrigenetic associations/interactions but with non-significant results in the evidence grading process.”

COMMENT

• Design: Please specify the population.

RESPONSE

Specified human studies, adult and pediatric.

COMMENT

• Design: As it is phrased, it looks like evidence was only included if it had a certain result, which is not appropriate for systematic reviews. All studies answering the a priori research question should be included and evaluated.

RESPONSE

Reworded to clarify that this is not what was done.

COMMENT

• Design: List databases searched and inclusion dates of articles.

RESPONSE

Added databases and dates.

COMMENT

• Results: All studies meeting inclusion criteria should be included.

RESPONSE

We agree and hope this is now clear with the revisions to the Design section (and throughout the full-text manuscript).

COMMENT

Introduction
• Objective: This should be stated in PICO format. What is the population. What specifically is the intervention or exposure? There are several questions being asked and answered and these should all be listed separately. Only use the word “effect” when referring to trials and “relationship” or “association” can be used for observational studies.

RESPONSE

We have revised our two specific objectives for clarity. We have clarified the PICO in brackets within our objectives.

COMMENT

Methods
• What was the start date of the search?

RESPONSE

Specified May 2020 in the Methods section.

COMMENT

• This helps clarify the intervention/exposure a little more, but it would be helpful to actually list out the separate PICO questions someplace. It seems more like a scoping review as is.
• A table that describes the inclusion/exclusion for each type of study/research question you are investigating would be helpful. There are a few different research questions that are being lumped together.

RESPONSE

Excellent suggestion – a table has been added to more clearly detail the PICO/PECO (Table 1).

COMMENT

• Evidence grading: This section is very confusing. There should never be studies that are “selected for grading” after studies have been included. All included studies are included in grading. Selecting studies after inclusion defeats the entire purpose of a systematic review. It makes no sense to choose the significant studies and then grade them. It is true that having two studies can result in high quality evidence, but that’s if those are the only studies answering the same question, they are RCTs, have large sample sizes, are consistent, etc. It is not ok to choose specific studies from included studies for grading. What if there were four studies that answered the question, two showed no relationship and two showed a relationship. We can’t simply ditch the two that found no relationship and say the relationship is positive. Maybe I am missing what the authors actually did here, in which case the process needs to be clarified.

RESPONSE

Again, we completely agree with you. We hope that our methods are now clearer with the revisions and thank you again for pointing out this confusion to us.

COMMENT

• It is completely against valid systematic review methods to “filter out evidence that would be deemed low or very low quality”. That is not a systematic review.

RESPONSE

To clarify, this is not what we did. We filtered out nutrigenetic associations/interactions that had not been replicated (but did not filter out all studies with non-significant findings), and therefore can be presumed as low or very low quality evidence according to GRADE (e.g. ZNT8, rs13266634, omega-3 intake and plasma HDL-c or TG assessed by Hosseini-Esfahani et al. 2017); we did not exclude all evidence that would be deemed low or very low quality from our evidence grading process. You will see that many of the studies cited in Table 2 (GRADE Evidence Profile) had non-significant findings, often leading us to grade down the level of evidence due to inconsistency in the studies’ results.

We understand that it may not be typical for systematic reviews in population-based nutrition to first conduct a broad search and then prioritize evidence grading topics, however the addition of genetic information in nutrigenetic research adds an extra layer of consideration in nutritional genomics. Because scientific validity of the body of knowledge in nutritional genomics is so poorly understood, we had to first broadly search the literature for all studies conducted on our topic and then prioritize the genes/outcomes selected for evidence grading. Given that GRADE identifies unreplicated findings as generally low or very low quality evidence, we systematically filtered out these unreplicated nutrigenetic associations/interactions from the evidence grading process.

In the “Evidence Grading” section of our methods in the original manuscript draft, we poorly used “study selection” where we should have used “nutrigenetic association/interaction selection.” Therefore, we have changed the wording throughout our manuscript for clarity. We of course did not want to filter out low quality studies, but rather nutrigenetic topics (associations/interactions) where the overall quality of evidence would be deemed low or very low based on at minimum lack of

replication. We had to prioritize topics for evidence grading given that several hundred different nutrigenetic associations/interactions were identified through our broad search and narrative review.

We hope that these revisions help to clarify our methods. We agree with all of the points you raised and would like to thank you for helping ensure that we are not implying that we only included studies with significant findings in the evidence grading process. This would indeed be a critical flaw. Should you have further recommended revisions we would be happy to review and consider these.

VERSION 2 – REVIEW

REVIEWER	Rozga, Mary Academy of Nutrition and Dietetics
REVIEW RETURNED	09-Nov-2021

GENERAL COMMENTS	Abstract • This search is now over a year old and should be updated before publication. • The research question isn't clear here. Can you state it in PICO form as is customary for SRs? • All included articles should be included in evidence grading. Picking and choosing which articles are included in grading defeats the purpose of a systematic review. • Line 50: Is evidence moderate or strong? Same for line 57. Introduction • This narrative sets up the study well. • Objective 1: "assessed nutrigenetic associations/interactions for genetic variants" does not seem like a comparator. I think what's missing is a lack of specificity. Because there is no specific genetic variant the authors are examining, there is no obvious comparator. This may not be specific enough for a systematic review question. • Objective 2: Same as above. When there is no specific genetic exposure being examined, there is no obvious comparator. • This systematic review is too broad. If authors only want to grade based on relationships that are reported in the literature, as scoping review should be done prior to systematic review to determine what is available in the literature. It is incorrect to pick and choose which studies are graded using the grade method after articles have been selected for inclusion. The systematic review question needs to be more narrow, because it is not really answerable in its current form. Methods • What is the start date for article publication that was included (example: articles published from Jan 2010 to Aug 2020 were included). I don't think the authors mean they only included three months of published data. • Same issue for the comparator. It seems like an answerable systematic review question would be something like: In participants with xxx genetic variant, what is the effect of omega 3 intake on lipid outcomes compared to participants with xxx genetic variant. As it is, the question is so big and unspecific, it doesn't seem directly answerable. It seems like the genetic variants of interest need to be defined in the PICO questions and inclusion criteria. I understand that some exposures are polygenic, but authors could still include these by saying "genetic variant x, or polygenic profiles including genetic variant x". This study casts a

	broad net, which is appropriate for scoping reviews, but not systematic reviews. • Lines 186-187: Why were only statistically significant results summarized? This goes against systematic review methods. Results for all included outcomes from all included studies should be summarized, whether or not they are significant. • As described in the prior review, it is incorrect to pick and choose which studies should be included in evidence grading. I appreciate that authors did not pick and choose based on statistical significance in this version, but any picking and choosing is incorrect. This is not how a systematic review is done. Is there a systematic review methodologist on the research team? Systematic prioritization for evidence grading based on if literature is present is incorrect. This should be determined prior to conducting the systematic review. If a specific question is asked, then it needs to be answered completely and graded. The problem in this systematic review is that there really isn't a specific question based on specific genetic variants of interest. Authors cannot select what specific interaction they are going to examine/grade based on if there is a potential for strong results after the articles are included. I think any systematic review methodologist would object to not including evidence in grading that is "likely to have weak evidence" once the systematic review is underway. Results • Lines 246-247: It is incorrect to select which results to report based on significance. All results of specified outcomes in the eligibility criteria should be included. The problem is that the SR "question" was just too broad to examine systematically without picking and choosing which studies to include, which is incorrect methodology. • I encourage the authors to read GRADEs criteria for marking down for imprecision. They used to say there needed to be at least 400 participants to not be marked down for imprecision, and they have since raised that to 800 participants. Therefore, for the row with 31-SNP Nutri-GRS and TG, which looks like the only relationship with high/strong evidence, there should be marking down for imprecision with just one study of 330 participants. Overall comments: I appreciate that the authors did not choose studies for grading based on significance. However, after inclusion, it is incorrect to pick and choose any studies at all to include in grading. ALL studies included should be graded. This is difficult in the current study, because the PICO question is too broad and doesn't specify exactly what is being compared (individuals with x genotype compared to y genotype). I would encourage authors to work with a systematic review methodologist to write specific, answerable PICO questions, include ALL studies that meet eligibility criteria, then grade all of those included studies to answer about strength of evidence for the results found.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 2

Dr. Mary Rozga, Academy of Nutrition and Dietetics

Dear Dr. Rozga,

Thank you again for taking the time to re-review the submitted manuscript. We appreciate your perspective on our methods and feel that both the methods you have suggested (completing a scoping review and then selecting a narrower topic for systematic review and evidence grading) and those that we have undertaken (completing a comprehensive systematic narrative review and then prioritizing topics for evidence grading) are both valid. All research approaches have limitations, and we welcome a discussion of the limitations you have identified with the methods we chose to undertake. We don't feel it would be appropriate to change our research questions at this stage, given that our research questions were identified a priori, as indicated in our PROSPERO registration. Given this, we have instead opted to add an important paragraph to our discussion detailing the limitations you identified as well as the methods that you have suggested so that future systematic reviewers can consider using these methods. We also highlight the limitations you have identified. Indeed, the methods you suggest would allow for all articles to be included in evidence grading (strength). However, this would also result in a far less comprehensive overview of a given nutrigenetic topic (limitation). Moreover, as we you probably know, we must keep in mind that the limitation of a scoping review is that many articles are often missed since a systematic search strategy is not used. If the ultimate goal of completing systematic reviews with evidence grading is to eventually guide clinical practice (when sufficient evidence exists), time and energy may be better prioritized towards nutrigenetic topics where evidence is strong. The methods we chose to use allowed us to identify topics that are more likely to have moderate or strong evidence, though we should note that we still found that most topics included in evidence grading only had low or very low levels of evidence. We hope that the revisions listed below allow us to "meet in the middle" with our individual perspectives on conducting systematic reviews with evidence grading in nutritional genomics. We hope you can see the strengths and limitations of both the methods you suggest and those that we used, both of which have value to the body of literature in nutrigenetics for their own unique reasons. We further hope that you can appreciate that it is unfortunately not feasible to complete evidence grading on every single gene-diet interaction/association identified in our review as there were hundreds identified in the included articles. Despite this, we hope you will agree that our review remains valuable to the field of nutrigenetics, and is a positive starting point for systematic reviews with evidence grading, that future researchers can now build off of. Thank you again for your time and consideration.

Abstract

COMMENT:

• This search is now over a year old and should be updated before publication.

RESPONSE:

Please note that this manuscript was originally submitted June 16, 2021. We understand that there have been delays in securing peer reviewers during the COVID-19 pandemic and this has led to some delays with the review of this manuscript; we also greatly appreciate you volunteering to serve as a peer reviewer on this paper given the current situation. Please note that the literature on the topic is sparse, so we are confident that we still cover the most important and the vast majority of papers published in the field. Moreover, the primary author of this paper is on maternity leave for the next

year. This being said, it is common however for such systematic reviews to have a year or longer delay between the search date and publication date. Still, we acknowledged this problem of delay between the end of a systematic review and its publication as a limitation of the paper. Please also note that our search was extremely comprehensive and remains a valuable contribution to the field of nutrigenetics given that systematic reviews with evidence grading in these field are few and far between (to our knowledge, this is only the second one).

COMMENT:

• The research question isn't clear here. Can you state it in PICO form as is customary for SRs?

RESPONSE:

We have revised the research question so that it is stated in PICO format. Please note however that this may need to be removed or abbreviated as we are now over the abstract word limitation for the journal.

COMMENT:

• All included articles should be included in evidence grading. Picking and choosing which articles are included in grading defeats the purpose of a systematic review.

RESPONSE:

We understand that we have taken a novel approach to the systematic review methods that is not "standard" in nutrition by first, broadly and systematically searching the literature, and next, prioritizing which nutrigenetic associations/interactions to include in evidence grading. To reiterate, we had to narrow down the nutrigenetic associations/interactions included in evidence grading because hundreds of combinations of genetic variants and plasma lipid/apolipoprotein outcomes were identified in our initial search. The most robust nutrigenetic associations/interactions were unknown prior to our review, therefore it was more beneficial to the field to complete a broad systematic search rather than narrowing in on a single SNP/nutri-GRS from the beginning. Given that the GRADE approach suggests that non-replicated results do not generally result in strong evidence, these non-replicated nutrigenetic associations/interactions were excluded from evidence grading in an effort to prioritize. For example, we did not conduct evidence grading for ZNT8, rs13266634, omega-3 intake and plasma HDL-c or TG (assessed by Hosseini-Esfahani et al. 2017) given that only one study assessed this. This approach allowed us to conduct a highly comprehensive narrative review on the topic, which to our knowledge has yet to be completed in nutrigenetics. We have indicated the lack of evidence grading for all of the hundreds of identified nutrigenetic associations/interactions as a limitation of the present review. We further noted that future systematic reviews with evidence grading may choose to select a specific gene-diet interaction/association to focus on, which would be a more efficient approach and perhaps more feasible for many research groups. It would also allow for all articles included in the narrative synthesis to undergo evidence grading.

COMMENT:

• Line 50: Is evidence moderate or strong? Same for line 57.

RESPONSE:

Evidence is “strong” – according the GRADE approach, the terminology “strong” is recommended to be used to describe evidence that is rated to be moderate or high quality. Therefore no changes were made.

Introduction

COMMENTS:

• This narrative sets up the study well.

• Objective 1: “assessed nutrigenetic associations/interactions for genetic variants” does not seem like a comparator. I think what’s missing is a lack of specificity. Because there is no specific genetic variant the authors are examining, there is no obvious comparator. This may not be specific enough for a systematic review question.

• Objective 2: Same as above. When there is no specific genetic exposure being examined, there is no obvious comparator.

• This systematic review is too broad. If authors only want to grade based on relationships that are reported in the literature, as scoping review should be done prior to systematic review to determine what is available in the literature. It is incorrect to pick and choose which studies are graded using the grade method after articles have been selected for inclusion. The systematic review question needs to be more narrow, because it is not really answerable in its current form.

RESPONSE:

We have further clarified what is meant by our comparator in the manuscript document; while it may not be a single specific SNP/nutri-GRS, it is still a valid comparator. We have added a paragraph on the limitations of the present review, which suggests that future researchers may choose a more specific research questions (e.g. focus only on a single SNP or single nutri-GRS), but it is at the same time counterproductive for articles using genome-wide approaches which are otherwise the studies most likely to give promising and non-biased results. We then consider that in the field, we already have a relatively narrow question. A narrower question as you do suggest, also has its own set of strengths and limitations. In an attempt to combine the standards of the fields of genetics and nutrition, our question represents, in our opinion, a scientifically acceptable and valid compromise. The same applies to the choice of including all studies for the specific priority nutrigenetic interactions/associations selected for evidence grading and therefore to complete a more comprehensive review with evidence grading compared to if we had selected only a single nutrigenetic interaction/association.

Methods

COMMENT:

• What is the start date for article publication that was included (example: articles published from Jan 2010 to Aug 2020 were included). I don't think the authors mean they only included three months of published data.

RESPONSE:

That is correct. To be comprehensive in our search, there was no start date – any article published prior to Aug 2020 was included. We have further clarified this.

COMMENT:

• Same issue for the comparator. It seems like an answerable systematic review question would be something like: In participants with xxx genetic variant, what is the effect of omega 3 intake on lipid outcomes compared to participants with xxx genetic variant. As it is, the question is so big and unspecific, it doesn't seem directly answerable. It seems like the genetic variants of interest need to be defined in the PICO questions and inclusion criteria. I understand that some exposures are polygenic, but authors could still include these by saying "genetic variant x, or polygenic profiles including genetic variant x". This study casts a broad net, which is appropriate for scoping reviews, but not systematic reviews.

RESPONSE:

While we took a novel approach to this systematic review, we were still able to answer our research questions; both Objective 1 and Objective 2 were answered in our manuscript. Again, we have detailed your perspective on how nutrigenetic systematic reviews could be conducted (and we agree that this approach is also valid) in the new paragraph added to the discussion section. It would not be appropriate to revise our PICO questions at this stage and outline all of the genetic variants that we identified in the included studies, and again it would not be appropriate for papers presenting results based on a genome-wide approaches. Given this, we hope that you will appreciate the new paragraph added to the discussion as a way to communicate the points you raised, and believe that this could help future systematic reviewers complete their reviews using the methods you propose.

COMMENT:

• Lines 186-187: Why were only statistically significant results summarized? This goes against systematic review methods. Results for all included outcomes from all included studies should be summarized, whether or not they are significant.

RESPONSE:

This was simply a decision made in order to most clearly communicate the results of the studies. Many included articles tested numerous nutrigenetic associations, most of which were non-significant (see, for example, Joffe et al. 2014). Because of the broad nature of our review article, adding all of

the non-significant results would diminish the readability of our tables by substantially cluttering the last column with all of the non-significant results. We specify in both the table footnotes and full-text manuscript that all other results for the listed genes/SNPs and lipid/apolipoprotein outcomes in the tables were non-significant. We also labelled the last column “summary of statistically significant study findings...” and link this title to the footnote to make sure this is absolutely clear.

COMMENT:

• As described in the prior review, it is incorrect to pick and choose which studies should be included in evidence grading. I appreciate that authors did not pick and choose based on statistical significance in this version, but any picking and choosing is incorrect. This is not how a systematic review is done. Is there a systematic review methodologist on the research team? Systematic prioritization for evidence grading based on if literature is present is incorrect. This should be determined prior to conducting the systematic review. If a specific question is asked, then it needs to be answered completely and graded. The problem in this systematic review is that there really isn’t a specific question based on specific genetic variants of interest. Authors cannot select what specific interaction they are going to examine/grade based on if there is a potential for strong results after the articles are included. I think any systematic review methodologist would object to not including evidence in grading that is “likely to have weak evidence” once the systematic review is underway.

Results

• Lines 246-247: It is incorrect to select which results to report based on significance. All results of specified outcomes in the eligibility criteria should be included. The problem is that the SR “question” was just too broad to examine systematically without picking and choosing which studies to include, which is incorrect methodology.

RESPONSE:

Please see comments above, which have addressed this point. Yes, co-author S. Desroches is a systematic review methodologist who was consulted throughout the review process from start to finish; she has published numerous systematic reviews in high impact journals (https://scholar.google.fr/citations?user=rm-aWU8AAAAJ&hl=fr). Again, both the methods you are suggesting and the methods we have chosen to use have their own strengths and limitations. An important strength of our methods is that we were able to conduct a highly comprehensive review of the literature, and include multiple nutrigenetic associations/interactions in the evidence grading. Had we selected just a single SNP/nutri-GRS, we would have missed other important nutrigenetic interactions. It might help to think of our systematic narrative synthesis as the scoping review portion that you are suggesting, except that it is systematic and thus less likely to miss studies.

COMMENT:

• I encourage the authors to read GRADEs criteria for marking down for imprecision. They used to say there needed to be at least 400 participants to not be marked down for imprecision, and they have since raised that to 800 participants. Therefore, for the row with 31-SNP Nutri-GRS and TG, which looks like the only relationship with high/strong evidence, there should be marking down for imprecision with just one study of 330 participants.

RESPONSE:

We have re-reviewed the GRADE handbook (https://gdt.gradepro.org/app/handbook/handbook.html), which does not specify that a sample size of 800 participants should be marked down for imprecision. In fact, sample size requirements for adequate power will vary depending on the study as different outcomes, for example, will alter sample size requirements. As such, the approach we used to evaluate imprecision is valid. No changes have been made to the imprecision evaluation on the basis of “800 participants.”

COMMENT:

Overall comments: I appreciate that the authors did not choose studies for grading based on significance. However, after inclusion, it is incorrect to pick and choose any studies at all to include in grading. ALL studies included should be graded. This is difficult in the current study, because the PICO question is too broad and doesn’t specify exactly what is being compared (individuals with x genotype compared to y genotype). I would encourage authors to work with a systematic review methodologist to write specific, answerable PICO questions, include ALL studies that meet eligibility criteria, then grade all of those included studies to answer about strength of evidence for the results found.

RESPONSE:

We appreciate you recognizing that grading all studies is difficult for the present review given the broad nature of the PICO question we selected. We would like to clarify that our methods for prioritizing studies were developed a priori; we prioritized studies on the basis of replication given that hundreds of nutrigenetic associations/interactions were identified so prioritizing was essential to the successful completion of this review. We did not pick and choose studies that we wished to grade at random. All PICO questions were answered in our review, and co-author S. Desroches (a systematic review methodologist: https://scholar.google.fr/citations?user=rm-aWU8AAAAJ&hl=fr) worked closely with the team and was consulted throughout the systematic review process. We hope our responses help to clarify the validity of our methods and hope that the revisions to our manuscript help to highlight the points that you have raised.

Reviewer: 2

Competing interests of Reviewer: None.

VERSION 3 – REVIEW

REVIEWER	Rozga, Mary Academy of Nutrition and Dietetics
REVIEW RETURNED	24-Jan-2022

GENERAL COMMENTS	This manuscript is much more clear than prior versions. I only have two major concerns. The first is that only significant results are included in the supplemental tables, which may be misleading. The second is the libery

VERSION 3 – AUTHOR RESPONSE

Reviewer: 2
Dr. Mary Rozga, Academy of Nutrition and Dietetics
Comments to the Author:

COMMENT:
This manuscript is much more clear than prior versions. I only have two major concerns.

The first is that only significant results are included in the supplemental tables, which may be misleading.

RESPONSE:
We have added further clarification about this in the manuscript text when we refer to these tables, so that we are not being misleading. Please see revisions to the first paragraph of the Results section. We would also like to reiterate our comment from the last round of revisions that this was simply a decision made in order to most clearly communicate the results of the studies. Many included articles tested numerous nutrigenetic associations, most of which were non-significant (see, for example, Joffe et al. 2014). Because of the broad nature of our review article, adding all of the non-significant results would diminish the readability of our tables by substantially cluttering the last column with all of the non-significant results. We specify in both the table footnotes and full-text manuscript that all other results for the listed genes/SNPs and lipid/apolipoprotein outcomes in the tables were non-significant. We also labelled the last column “summary of statistically significant study findings...” and link this title to the footnote to make sure this is absolutely clear.

COMMENT:
The second is the liberty of adding "biological mechanisms" to the GRADE summary of findings table, to allow the upgrading of evidence if there was a known biological mechanism but this was not discussed in the methods. Upgrading for biological plausibility is not part of the GRADE method as I understand it. From a methods perspective, an SR isn't rating evidence according to if it's possible there may be an effect, it's rating the evidence as it actually is. I suggest removing this addition to the grading/summary of finding table or reporting throughout the manuscript that the grading method was "based on" or "adapted from" the GRADE method. I also suggest discussing this adaptation in the methods section.

RESPONSE:
We have revised the paper to indicate the adapted/modified GRADE method/approach. We further clarified this in the methods section; thank you for bringing it to our attention that this was not indicated in the methods but only in the discussion. Many thanks again for volunteering to thoroughly peer review this paper.