Author’s response to reviews

Title: Lack of efficacy of pomegranate supplementation for glucose management, insulin levels and sensitivity: evidence from a systematic review and meta-analysis

Authors:

Haohai Huang (haohaihuang@hotmail.com)
Dan Liao (26312526@qq.com)
Guangzhao Chen (gzchen90@126.com)
Honglang Chen (915475709@qq.com)
Yongkun Zhu (379805596@qq.com)

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Author’s response to reviews:

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Dear Prof. Ley,

On behalf of my co-authors, we thank you very much for giving us an opportunity to revise our manuscript entitled "Lack of efficacy of pomegranate supplementation for glucose management and insulin sensitivity: evidence from a pooled analysis (NUTJ-D-17-00177)". We also appreciate the reviewers very much for their positive and constructive comments and suggestions on our manuscript.

We have studied reviewers’ comments carefully and tried our best to make revisions which are marked in red in the revised manuscript. Attached please find the revised version, which we would like to re-submit for your kind consideration. In addition, point-by-point responses to the comments are listed as following:

Responds to the reviewers’ comments:

Reviewer #1:

The present meta-analysis investigated the effect of pomegranate on parameters of glucose metabolism such as fasting blood glucose (FBG) and fasting blood insulin (FBI). Only
randomized controlled trials (RCTs) were considered for data analysis. It is concluded that pomegranate did not show favorable effects on the improvement of glucose and insulin metabolism.

General comments

Some meta-analyses about the effect of pomegranate on inflammatory process and lipid parameters have already been published. However, a meta-analysis concerning the effect of pomegranate on parameters of glucose metabolism is still lacking. The manuscript is well written and the meta-analysis is well done. This reviewer has only a few minor comments, which are outlined below.

Minor comments

1) Abstract, line 78: The second sentence of this line should be deleted and the first sentence should be changed as follows: Overall, significant heterogeneity was detected for FBI and HbA1c, but subgroup analysis could not identify factors significantly influencing these parameters.

Response: Thanks for your suggestions and cautious correction. Accordingly, we have rewritten this sentence in the revised manuscript (Lines 79-81).

2) Line 114: correct 'etabolism' into 'metabolism'.

Response: Thanks for your careful review and cautious correction. Accordingly, correction has been made in the revised manuscript (Line 118).

3) Methods section, subheading 'Data sources and search strategy' (lines 124-134): Was the meta-analysis registered at PROSPERO?

Response: Thanks for your comments. Our systematic review and meta-analysis was not registered prospectively at the International Prospective Register of Systematic Reviews (PROSPERO). We are going to register this systematic review and meta-analysis with the PROSPERO in the near future.

4) Lines 175: Change 'previously' into 'pre-specified'.
Response: Thanks for your careful review and cautious correction. Accordingly, correction has been made in the revised manuscript (Lines 201 and 342).

5) Line 255: Delete the word 'meanwhile'.
Response: Thank you for your recommendation. Accordingly, we have deleted this word in the revised manuscript (Line 276).

6) Line 263: Delete the word 'furthermore'.
Response: Thank you for your recommendation. Accordingly, we have deleted this word in the revised manuscript (Line 285).

7) Line 271: Delete the word 'also'.
Response: Thank you for your recommendation. Accordingly, we have deleted this word in the revised manuscript (Line 293).

8) Lines 273, 279 and 280: Use only two digits for the presented P-values throughout the manuscript.
Response: Thanks for your suggestions. Accordingly, we have made some modifications in the revised manuscript. All P-values throughout the manuscript were used in two digits (Lines 295, 301 and 302).

9) Discussion, line 283: At the beginning of the discussion section, one or two sentences should briefly summarize major findings of the present analysis. For example, the last sentence of line 312 up to the first sentence of 314 can be placed at the beginning of the discussion section. However, replace in line 315 the word 'previous' by the word 'pre-specified'.
Response: Thanks for your insightful suggestions and cautious correction. Following your advice, we have made some modifications in the revised manuscript (Lines 307, 308 and 342).

10) Lines 293-307: Since the present meta-analysis could not show a statistically significant effect of pomegranate on parameters of glucose metabolism, this paragraph can be shortened.
Response: Thanks for your insightful suggestions. Accordingly, we have made some modifications in the revised manuscript (Lines 328-334).

11) Lines 342-357: They should also add the limitation that the number of included RCTs with DM patients was small. Therefore, one can only expect a small effect of pomegranate on parameters of glucose metabolism.

Response: Thanks for your insightful suggestions. Due to the limited number of DM patients included, the overall effects of pomegranate on parameters of glucose metabolism are inconclusive; further adequately powered studies investigating the effects of pomegranate on biomarkers of glucose metabolism in adults with DM are needed. Accordingly, we have commented on these points in the LIMITATIONS section of our revised manuscript (Lines 379-382).

Reviewer #2:

This study is a systematic review and meta-analysis of studies exploring the effect of pomegranate products on glycemic control, insulin levels, and sensitivity.

Diabetes mellitus/glucose intolerance is a chronic disease in which various alternative treatments of unproven efficacy are often tried either alone or in combination with other medications. There have been claims about the efficacy of pomegranate products which are put to scrutiny in this study, which makes the study relevant.

Title:

1) The PRISMA guidelines state that manuscript should be identified as meta-analysis and systematic review, please include that in the title. As fasting blood insulin was one of the primary outcomes, it should be included in the title.

Response: Thanks for your insightful suggestions. We have incorporated these points into the Title page.

Abstract:

2) Abstract is succinct and summarizes the study, however it should be updated to include the changes that I have described below.
Response: Thanks for your insightful suggestions. Following your advice blow, we have made some modifications in the Abstract section of the revised manuscript.

Background:

3) While the scope of problem of diabetes mellitus and importance of glycemic control is described appropriately, majority of studies included in this trial are not done on diabetic individuals. The background should include more details about the insulin resistance and primary prevention in healthy individuals.

Response: Thanks for your insightful suggestions. Subjects with impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) are usually considered to have a high future risk of developing diabetes. Moreover, prospective cohort studies in subjects without diabetes have also revealed that increased insulin resistance worsened glycemic control and contributed to the development of type 2 diabetes mellitus (T2DM). Appropriate management of hyperglycemia is thought to decrease the complications and morbidity of diabetes. Given its high disability rate and mortality, identifying modifiable lifestyle factors is important in primary, secondary, and tertiary prevention in patients with diabetes and healthy people. Accordingly, we have incorporated some key points into the BACKGROUND section (Lines 99-105) of the revised manuscript.

4) The sentence, Line 113, "Encouraging findings from experimental research….." needs references to more than one study, so does the next sentence, line115, "several observational studies…” in which references to all the original studies should be provided.

Response: Thanks for your insightful suggestions. We have cited some relevant original studies in these sentences (Lines 119 and 120).

5) The next sentence, Line 116, should be rephrased to "However, the effects of pomegranate on insulin and glucose metabolism in humans are inconsistent and their precise role in the management of hyperglycaemia has not been fully established". The sentence also need references.

Response: Thanks for your insightful suggestions. We have cited the relevant references in these sentences (Line 122).
6) The last sentence describes the objective of the study. This should be done under a separate sub-heading and primary and secondary objectives need to be more explicitly described with reference to participants, interventions, comparators, and outcomes (PICO)

Response: Thanks for your insightful suggestions. Accordingly, we have made some modifications in the revised manuscript (Lines 123-128).

Methods:

7) Is the protocol for review available to public and were there any deviations from the protocol?

Response: Thanks for your comments. We are going to register this systematic review and meta-analysis with the International Prospective Register of Systematic Reviews (PROSPERO) in the near future. This protocol file is not publicly available until the review is complete and publish. There are not any deviations between the protocol and review methods of the present study.

Eligibility Criteria:

8) The eligibility criteria should precede search strategy in the manuscript.

Response: Thanks for your comments. The eligibility criteria section is now list before the search strategy section. Accordingly, we have made some modifications in the revised manuscript (Lines 131-141)

9) The eligibility criteria should be described under separate subheadings of types of study, participants, intervention and outcome measures. The inclusion criteria do not define the type of participants which must be included.

Response: Thanks for your insightful suggestions. We have defined the type of participants in the inclusion criteria. We regarded studies as eligible for inclusion if they met the following criteria: 1) Study participants: adult male and female participants (age ≥18 y) with or without comorbidities (such as hypertension, diabetes, and peripheral arterial diseases) were included. 2) Types of interventions: participants needed to have specifically ingested the pomegranate interventions (no matter what type and regimen applied) ≥1 week. Studies in which pomegranate was combined with other interventions (e.g. taking glucose lowering drugs) were included when the control group performed the same treatment. 3) Comparators: placebo or other interventions; 4) Outcome measures: studies reported data on at least one of the following endpoints: fasting blood glucose (FBG), fasting blood insulin (FBI), glycated hemoglobin (HbA1c), and
homeostatic model assessment of insulin resistance (HOMA-IR). In addition, the initial or endpoint values for outcomes or their difference and their SD or SE or 95% CI of each group were available. 5) Study design: study was an RCT in human with either a parallel or crossover design. Accordingly, we have made some modifications in the revised manuscript (Lines 132-141).

Data Sources and Search strategy:

10) There is inadequate description of the search strategy. Please mention who was involved in the creation of search strategy, and was it peer reviewed?

Response: Thanks for your insightful suggestions. In our present study, two investigators (H.C. and D.L.) independently performed the literature search. Any disagreements were resolved by discussion and consensus. Accordingly, we have commented on these points in our revised manuscript (Lines 159-162).

11) Please provide a draft of search strategy of at least one database either in the main text or as an appendix.

Response: Thanks for your insightful suggestions. The structured draft of search strategies used the following search key words and Medical Subject Headings (MeSH) terms: (pomegranate OR Punica) AND (glycemic control OR glycaemic control OR glucose control OR glycaemic OR glucose OR blood sugar OR blood glucose OR fasting plasma glucose OR FBG OR glucose tolerance OR insulin resistance OR insulin OR blood insulin OR fasting blood insulin OR insulin sensitivity OR FBI OR Hemoglobin A1c OR HbA1C OR glycated hemoglobin OR glycolated haemoglobin OR homeostatic model assessment of insulin resistance OR HOMA-IR OR diabetes mellitus OR diabetes) AND sensitivity RCT filters (the specific and sensitive strategies developed to ensure optimal collection of RCTs in electronic searches). The search was restricted to English-language publications and the reports of clinical trials conducted in human subjects. Accordingly, details of search strategies has been described in the "Data sources and search strategy" main text of our manuscript (Lines 149-157).

12) Please mention if the trial registers were searched for ongoing trials and whether PROSPERO was searched for ongoing or recently completed related systematic reviews. Was the search restricted to studies in English language?

Response: Thanks for your insightful suggestions. Following your advice, we further search the Google Scholar, PROSPERO (https://www.crd.york.ac.uk/PROSPERO/) and ClinicalTrials.gov
(https://clinicaltrials.gov/) for the ongoing or recently completed related systematic reviews. However, we failed to identify any eligible studies for inclusion. Our search was restricted to English-language publications and the reports of clinical trials conducted in human subjects. We have commented these points in the revised manuscript (Lines 148-149, and 156-157).

Data Extraction:

13) The description of data extraction process is inadequate. Please describe the mechanisms that were used to manage data in the review including use of computer programs or software if any. Please describe the selection process, who was involved in the selection and data extraction and how were the differences (if any) resolved.

Response: Thanks for your comments. In our present study, data extraction was performed by D. L. and was confirmed independently by two other authors (H.H. and H. C.). Extracted data were entered into a predefined standardized Excel (Microsoft Corporation, USA) file. We also sought supplementary files of included trials or contacted corresponding authors to verify extracted data and request the missing data. All values were captured as mean ± SD. When SDs were not directly available, they were calculated from SE or 95% CI according to the Cochrane Handbook for Systematic Reviews of Interventions [Higgins JP, et al. 2011]. Accordingly, we have commented on these points in the "Data Extraction" section of our revised manuscript (Lines 165-168 and 175-177).

Reference


Assessment of methodological quality:

14) The risk of bias tool used to assess the methodological quality is robust and used appropriately, however, there is indirectness of evidence in terms of differences in population and differences in intervention, due largely to a lenient inclusion criteria, there is also inconsistency in effect and effect size across studies. While subgroup analysis and meta-regression will help address some of these issues, a tool like GRADE that helps rate quality of evidence for each outcome across studies taking into account the above factors is needed to help make more objective decisions about the results.

Response: Thanks for your insightful suggestions. Following your advice, two authors (H. H. and D. L.) independently evaluated the quality of evidence for primary and secondary outcomes
according to GRADE methodology for risk of bias, inconsistency, indirectness, imprecision, and publication bias; rated as very low, low, moderate, or high. If disagreements occurred between the two reviewers, a third author would make decision through discussion. Summary tables were constructed using the GRADE Profiler (GRADEpro, version 3.6). The GRADE evidence profiles for the primary and secondary outcomes are presented as Additional file 1. The GRADE Working Group grades level of evidence is high for FBG and HbA1c; and moderate for FBI and HOMA-IR. Accordingly, we have commented on these points in our revised manuscript (Lines 189-194, 302-304, and Additional file 1).

Statistical analysis

15) Choosing a meta-analysis model based on statistical heterogeneity is not recommended [1]. There exist significant clinical and methodological differences in the pooled studies to warrant the use of random effect model only.

Response: Thanks for your insightful suggestions. Following your advice, we pooled outcome data using a random-effects model accounting for clinical heterogeneity. In our revised manuscript, the effect sizes of defined outcomes were expressed as weighted mean difference (WMD) and 95% confidence interval (CI) by using a random-effects model. Accordingly, we have made some modifications in the revised manuscript (Lines 200-201, 258 and 274).

Results:

Identification of studies:

16) The boxes in figure one needs to be rearranged. The text in line 199 need to be revised so it describes what's represented in the figure.

Response: Thanks for your careful review. We have re-written the “Identification of studies” section following the flow diagram in Fig.1. Briefly, the initial search yielded 139 potentially relevant citations. After the removal of duplicates, 102 titles and abstracts were screened; of these, 78 were excluded because they were clearly irrelevant to our meta-analysis. The full-text publications were obtained for the remaining 24 articles. A total of 10 articles were subsequently excluded for the reasons listed in Fig.1. Subjects in one study were also divided into 2 subgroups on the basis of different doses of pomegranate ellagitannin extract consumption used (710 mg/day intake subgroup and 1420 mg/day intake subgroup). The work conducted by Fuster-Munoz et al. was also separated into the pomegranate juice intake subgroup and pomegranate juice diluted 1:1 with water intake subgroup. Finally, a total of 16 RCTs that met our inclusion criteria were included in the present pooled analysis. Accordingly, correction has been made in the revised manuscript (Lines 223-232).
17) Please explain why this 2013 study by Banihani et al was not included [2].

Response: Thanks for your comments. The study conducted by Banihani et al. was excluded because of this study was not an RCT.

Study characteristics

18) Please mention the names of studies studying the outcome of interest in the text.

Response: Thanks for your careful review and suggestions. The relevant studies which reported the outcome of interest have been cited in our revised manuscript (Lines 239-240).

Overall effect of pomegranate on glucose control and insulin sensitivity

19) The study was Cerda et al does not mention fasting blood glucose as the outcome measure, were the authors contacted to confirm that glucose measured was actually fasting?

Response: Thanks for your careful review. In the study conducted by Cerda´ et al, the glucose concentration was regarded as fasting blood glucose; since the author described that the blood extraction was carried out in the morning before breakfast. Meanwhile, we have E-mail the corresponding author to confirm this outcome measure.

20) The subgroup analyses and sensitivity analysis is adequate and takes into account most confounders.

Response: Thanks for your encouraging comments on the manuscript.

Discussion:

21) Discussion should provide more information on how pomegranate came to be used in the prevention and management of diabetes including more references for studies in human and animals.

Response: Thanks for your insightful suggestions. Accordingly, we have reviewed the relevant studies and incorporated some key points into the Discussion section (Lines 325-334) of the revised manuscript. The potential effects of pomegranate consumption in the prevention and management of diabetes can be explained by following hypotheses: study conducted by Huang et
al. demonstrated that treatment with punica granatum flower extract could enhance the mRNA expression of cardiac PPAR-γ and restored mRNA expression of the cardiac glucose transporter 4 (GLUT4). Later reports have shown that pomegranate/extracts affect the diabetic condition by antagonizing the damaging effects of pro-oxidants and reducing oxidative stress and lipid peroxidation. Pomegranate flowers also ameliorate T2DM in ZDF rats by enhancing the expression of hepatic genes involved in fatty acid oxidation (e.g. acyl-CoA oxidase, and carnitine palmitoyl-transferase-1). Furthermore, pomegranate extracts is beneficial in controlling glucose homeostasis in humans by suppressing the activation of NF-κB, neutralizing the generated reactive oxygen species and the expression of tumor necrosis factor-α, which finally diminish the development of T2DM.

22) Include examples of other herbal medications used in diabetes, and discuss if their use can cause any potential harm.

Response: Thanks for your insightful suggestions. In recent decades, dietary micronutrients or herbal medications interventions were generally used to improve glycemic control and other CVD risk factors among individuals with T2DM, and the public has embraced their efficacy and safety. A recent meta-analysis reported that consumption of cinnamon is associated with a statistically significant decrease in levels of fasting plasma glucose, total cholesterol, LDL-C, and triglyceride levels, and an increase in HDL-C levels. Potential side effects reported to be associated with cinnamon were hepatotoxicity, decreased platelet counts, increased the risk of bleeding, and markedly increased allergy/hypersensitivity. However, human studies suggested that no significant side effects were seen with cinnamon use. Hausenblas et al. found that resveratrol supplementation was more effective than placebo/control, were identified for systolic blood pressure, HbA1c, and creatinine. The incidence of side effects was very small, not different than placebo, and no major adverse events were reported. Accordingly, we have commented on these points in our revised manuscript (Lines 315-324).

Figures:

23) Figure 1 should be corrected as above

Response: Thanks for your comments. The Fig.1 has been revised as the comments above.

24) Figure 3 should include number of participants for each study, their mean and SD (or as applicable). Each forest plot should be labeled to indicate the model of meta-analysis used.
Response: Thanks for your comments. The models of meta-analysis used for the outcomes have been labeled in the forest plots. Since our forest plots were generated by using STATA (version 12; StataCorp, College Station, TX), the number of participants for each study, their mean and SD were not present in the Figures. However, the number of participants for each study can be found in Table 1.

Language errors:

25) There are several language errors throughout the manuscript that need editing.

Response: Thanks for your cautious correction and useful suggestions. The revised manuscript has been edited and proofread by a native English speaker. I hope that the revised manuscript is now acceptable for publication in the Nutrition Journal.

REFERENCES


Reviewer #3:

This is a meta-analysis for RCT evaluating pomegranate supplementation effects on blood glucose management. Publication bias, subgroup analyses and sensitivity analysis were also conducted, which did not change the conclusion that pomegranate intake did not show a notably favorable effect on the improvements of blood glucose and insulin management. The methodology is fine, however, too many mistakes authors need to be more careful and some issue need to be addressed (see below).

1. In Abstract, there were some statements inconsistent with the main text.

(1) HbA1c (WMD, -0.03 mmol/L; 95% CI, -0.29 to -0.22; P=0.79), these values were different from L235 and Figure 3.
Response: Thanks for your careful review and kind reminding. The correct value of HbA1c should be (WMD, -0.11 %; 95% CI, -0.39 to 0.18; P=0.46). Accordingly, we have made some modifications in the revised manuscript (Line 79).

(2) The units used for WMD of insulin, HOMA, and HbA1c were incorrect. As authors stated all values for insulin were converted into "μIU/mL".

Response: Thanks for your careful review and kind reminding. The correct units used for WMD of insulin and HbA1c should be “μIU/mL” and “%”, respectively. Accordingly, we have corrected the units for these outcomes throughout the manuscript.

(3) "Significant heterogeneity was detected for FBI and HbA1c." According to Results, heterogeneity occurred in FBI and HOMA.

Response: Thanks for your careful review. We have checked our manuscript carefully. Yes, this sentence in the Results section was wrong due to my carelessness. The correct description should be “significant heterogeneity was detected for FBI and HOMA-IR”. Accordingly, we have corrected these points in the revised manuscript (Line 80).

2. The nature and composition of pomegranate juice, pomegranate seed oil, and pomegranate extract are quite different. Authors should elaborate on the major compounds in PJ, PE and PSO. The statement of "Pomegranate (Punica granatum L.) contains a high concentration of total polyphenols (e.g. ellagic acids, gallotannins, anthocyanins and other flavonoids)" in L108 is not informative since above mentioned compounds might not be found in PSO. In contrast, PSO is famous for a conjugated fatty acid, punicic acid. This should be indicated. Regarding PE, which part of plant was extracted? What are their major components?

Response: Thanks for your insightful comments and suggestions. Accordingly, we have reviewed the relevant studies and incorporated some key points into the BACKGROUND section (Lines 108-114) of the revised manuscript. Pomegranate (Punica granatum L.) has a high content of antioxidants and bioactive polyphenols, being widely investigated for its antioxidant, anti-inflammatory, anti-atherogenic, and anti-hyperglycemic effects. Fresh pomegranate juice (PJ) is rich in phenolic acids (including gallic acid, caffeic acid, chlorogenic acid, ferulic acid, and coumaric acids), non-phenolic acids, citric acid, succinic acid, malic acid, oxalic acid, and ascorbic acid. Pomegranate seed oil (PSO) consists of about 80% conjugated linolenic acid (9-cis, 11-trans, 13-cis) octadecatrienoic acid or punicic acid. Pomegranate extract (PE) contains abundant anthocyanins, punicalin, pedunculagin, punicalagin, gallagic acid and ellagic acid. In the present analysis, 3 included studies applied pomegranate fruit extract as treatments.
3. L114, "glycemic etabolism" mis-spelling?

Response: Thanks for your careful review. Accordingly, correction has been made in the revised manuscript (Line 118).

4. In M&M, it is confusing for trials with multiple intervention groups, as L155-166 stated "we grouped together all the experimental groups and compared them with the control group" However, in L202-205, it seems intervention groups with 2 different doses were regarded as two separate trials. Please clarify.

Response: Thanks for your careful review and useful comments. For trials with more than one intervention group (eg, with different doses of pomegranate), multiple comparisons were considered. The work conducted by Fuster-Munoz et al. applied 2 different doses of PJ consumption, so we divided this study into 2 trials for pooled analysis. Accordingly, we have commented on these points in our revised manuscript (Lines 173-174, 228-231).

5. In Result, Identification of relevant studies, the information provided was not clear and enough:

(1) In Figure 1, "additional records identified through other sources" (what sources? should be indicated).

Response: Thank you for your insightful question. Additional records (n=2) were identified from the reference list of included publications and other potentially eligible trials search. Accordingly, this information has been added to the Flow Diagram in Figure 1.

(2) "72 were excluded either because of duplication or because they were irrelevant to our meta-analysis (this description was not matched with Figure 1). In Figure 1, how come n=32 turns to n=24 after removing n=72?"

Response: Thanks for your careful review. The initial search yielded 139 potentially relevant citations. After the removal of duplicates, 102 titles and abstracts were screened; of these, 78 were excluded because they were clearly irrelevant to our meta-analysis. The full-text publications were obtained for the remaining 24 articles. A total of 10 articles were subsequently excluded for the reasons listed in Fig.1. Subjects in one study were also divided into 2 subgroups on the basis of different doses of pomegranate ellagitannin extract consumption used (710 mg/day intake subgroup and 1420 mg/day intake subgroup). The work conducted by Fuster-
Munoz et al. was also separated into the pomegranate juice intake subgroup and pomegranate juice diluted 1:1 with water intake subgroup. Finally, a total of 16 RCTs that met our inclusion criteria were included in the present pooled analysis. Accordingly, correction has been made in the revised manuscript (Lines 223-232).

6. Table 1, some values in column of "Mean age" seems to be range, not mean. I recommend to provide "mean" and also "range", if they were available. The same for BMI. The nature of placebo (starch, olive oil…?) would be better to provide.

Response: Thank you for valuable suggestion. Among the included studies, the values of age and the BMI were captured as means ± standard deviation, except the studies conducted by Heber et al. and Hosseini et al. (due to the missing data). These values in these studies were provided as the range (Table 1). Only 5 studies provided the ingredients of placebo used during the study period (Table 2).

7. In Results, L224-225, It was unclear "When we judged all three domains to have a low risk of bias, we designated the trial as having a low risk of bias."

Response: Thank you for your insightful question. The assessment of quality characteristics used the following criteria: 1) sequence generation of allocation; 2) allocation concealment; 3) masking of participants, personnel; 4) blinding of outcome assessors; 5) incomplete outcome data; 6) selective outcome reporting; and 7) other sources of bias. Each item was judged as low, unclear, or high risk of bias, based on whether the level of bias in domains may have led to material bias in the outcomes of interest. Trials with high risk of bias for any one or more key domains were considered as at high risk of bias; while trials with low risk of bias for all key domains were considered as at low risk of bias; otherwise they were considered as at unclear risk of bias. Overall, 5 trials were categorized as at low risk of bias, 2 as at high risk of bias, and 9 as unclear. Accordingly, we have made some modifications to our conclusion in the revised manuscript (Lines 185-187 and 251-253).

8. The quality of Fig 3 is not good.

Response: Thanks for your comment. The image resolution of Fig. 3 in our manuscript is reach 300 dpi at the final size. We hope that the quality of the Fig. 3 is now acceptable for publication in the journal.
9. Shorten L247-258, there was no need to repeat the data in Table 2. Moreover, the data for parallel and crossover design list in text were not matched with those in Table 2.

Response: Thanks for your insightful suggestions. Following your advice, the summary of the subgroup analysis results were shortened in the “Subgroup analyses and sensitivity analysis” section of our revised manuscript. Meanwhile, we have examined the manuscript carefully and ensure the accuracy and integrity of the data (Lines 274-282).

10. L259-260, "the subgroup analyses indicated that differences in study design, type of intervention, …. did not appear to significantly influence pooled mean differences in FBI concentrations." This was not true. Table 2 shows there was a significant effect of supplement on FBI within PJ <250 ml/g subgroup.

Response: Thanks for your careful review and comments. In a subgroup analysis stratified by type of intervention, the pooled result shown that pomegranate supplementation did not significantly affect the level of FBI (WMD, 2.86 μIU/mL, 95% CI, 0.00 to 4.88; P=0.05) in subjects who consumed consumption <250 ml of PJ daily. In the “Statistical analysis” section, authors stated that a P value of < 0.05 was considered statistically significant for all analyses. Base on the summary of subgroup analyses, the authors therefore draw the conclusion that the differences in study design, type of intervention, baseline BMI, and healthy status of the participants did not appear to significantly influence pooled mean differences in FBI concentrations (Table 2).

Once again, thank you very much for your comments and suggestions. Please also advise if you need any additional information for the revised manuscript. Thank very much for your consideration, and look forward to hearing from you soon.

Yours sincerely,

Haohai Huang, M.D.

Department of Clinical Pharmacy, Dongguan Third People's Hospital, Affiliated Dongguan Shilong People’s Hospital of Southern Medical University