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The effects of trans-resveratrol on insulin resistance, inflammation, and microbiota in men with the metabolic syndrome: a pilot randomized, placebo controlled clinical trial

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The Effects of Trans-Resveratrol on Insulin Resistance, Inflammation, and Microbiota in Men with the Metabolic Syndrome A Pilot Randomized, Placebo Controlled Clinical Trial
Journal of Clinical and Translational Research

Dear Dr. Heger,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript.Also, please ensure that the track

changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Oct 18, 2018.

To submit a revision, go to https://jctres.editorialmanager.com/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: Dear Authors,

Thank you for allowing me to review this manuscript, which lays down further information about the potential role of resveratrol as a therapy for treating the metabolic syndrome by presenting the results of a pilot RCT. I have specific comments regarding sections of the text below.

ABSTRACT
Please refer to "coronary heart disease" or "coronary artery disease" rather that "coronary disease"

After "The polyphenol resveratrol (RES) is believed to improve glucose homeostasis and insulin resistance by activating sirtuin, which acetylates and co-activates downstream targets." please add a few words to summarize how acetylating and co-activating downstream targets improve glucose homeostasis.

RELEVANCE FOR PATIENTS
This is a pilot study and while the significant changes seen in the small sub-group offer support to pursue research in this direction, they are not sufficient to support the use of resveratrol in patients with the metabolic syndrome. In addition, the cost of such a large dose of resveratrol is not necessarily low nor cost-effective given that the cost of first-line diabetes medication such as metformin is also low and has been shown to have a much greater effect on glucose homeostasis in multiple large-scale clinical trials that what this study has shown. Please modify this section to reflect the degree of uncertainty in applying these results to patients, and remove referece to the low cost of resveratrol.

INTRODUCTION
Page 4, line 45: This sentence should be revised. Resveratrol is more commonly extracted from the Japanese knotweed plant for dietary supplements. Grapevine leaves also contain significant amounts of resveratrol and may be a better source for extracts as they are produced in large amounts as waste products from the grape growing industry. Other natural sources also include

certain berries and peanuts.
Page 4, line 51: The Zamora reference is not provided in the reference list
Page 5, line 41-43: resveratrol is modified by the gut microbiota primarily in the large intestine, and not the stomach. Please revise use of "gastrointestinal tract"
Page 5, line 48-51: Please describe how resveratrol affects TMAO production and its relevance for MetS.

MATERIALS AND METHODS
Page 7, line 46-51: The description of screening results is different to what is reported in the Results section. Perhaps this paragraph can be taken out and any extra information added to the repeated information in the results section? In any case, the clamp procedure was listed as an inclusion criteria, yet subjects appear to have been included before this procedure had taken place. Perhaps it was a secondary screening procedure? Or a baseline procedure that was not part of the inclusion criteria?
Page 7, line 58: It would be helpful to describe the race/ethnicity of the non-Caucasians if possible, especially given differences in diet and risk of diabetes in various ethnic groups that may contribute to null results in non-Caucasians.
Page 8, line 57-60: Please describe the type of resveratrol in the product and its source.
Page 9, line 26-29: Please state whether subjects' weight was including clothing
Page 10, line 36-40: Please state when the fecal samples were obtained. Often the sampling occurs at home and the samples are brought in frozen to the center, but it is possible that fecal samples were taken during the study. Please clarify.
Page 15, line 4-7: the sample size calculation indicated that 45 subjects in each group were required, yet only 14 were finally included. Please comment on the effect of the final sample size on the relevance of the results.

Additional comment: please consider adding a description of how the subjects were randomized into the study, and how the investigational product was kept double-blinded to receive the maximum score on the Jadad scale (Jadad et al., 1996).

RESULTS
Page 17, line 10-15: The description of the flow of subjects through the study does not match up with the flow chart in figure 1. The description states that 14 subjects were randomized into each group, however the flow chart indicates that 17 and 16 were randomized, and there were 5 drop outs. The description claims that there were 34 eligible subjects, but figure 1 states that 33 were eligible. As insulin resistance was considered to be an inclusion criterium, I would normally assume that the clamp procedure was performed before randomization, however the way that it is described and appears in the flow chart indicates that the clamp was performed after randomization. Please correct, or address this departure from clinical trial norms.

DISCUSSION
Page 21, line 19: The results are inconclusive, not negative.
Page 21, lines 51-54: Bode does not investigate the effect of race, therefore state explicitly here that the results could be a chance finding due to high inter-person variability in RES absorption or metabolism. An additional point to make here is that there may be more heterogeneity in diets in the non-Caucasian group (presumably consisting of different race-ethnicity groups such as

African Americans and Asians, who have distinctive dietary patterns), leading to greater variability in measures of glucose homeostasis in the non-Caucasian group.
Page 23, line 9: These were null findings, not negative findings.

Reviewer #2: Walker et al. submitted an interesting manuscript on the effect of resveratrol on obese men with metabolic syndrome, and the features associated with MetS. RES has been investigated extensively in pre-clinical settings, and has been suggested to positively impact several biological systems, including glucose metabolism. This study seems well planned and very comprehensive, including clamp, glucose tolerance test, resting energy expenditure, adipose tissue biopsy, and fecal microbiota. The authors report only a marginally altered glucose homeostasis overall, however, in subanalyses, this seems to be driven by a more convincing effect within a very small group of Caucasians, while non-caucasians had no effect. Fecal microbiota were also affected differently according to race. Other features of the MetS were not affected by RSV.

I do have some questions and concerns for the authors to reply.

1)
It is unclear if the study participants were "admitted" 24-hours/day at the metabolic unit duing the entire 35 day study? This would provide extremely controlled conditions, which overall is good, but will certainly affect comparisons intra-individually pre- and post- treatment. The randomized design will of course make up for this.

2)
The differences between the Caucasians and non-caucasians in relation to response to RES on glucose homeostasis are described (within the results section) as differences comparing the races: "As shown in Table 4B, there were highly significant effects on insulin resistance as measured by the insulin clamp technique in Caucasian when compared to non-Caucasian subjects ($p<0.001$). Furthermore, the 120 minute end point glucose level and the glucose concentration area under the curve during the glucose tolerance test were significantly lower in Caucasian subjects (Table 4B). At baseline, these two groups did not differ in age, BMI or the number of components of the metabolic syndrome (Data not shown)."
The tables showing the data (table 4a and 4b) instead reveal comparisons between the treatment groups (RES vs placebo) when divided in Caucasians and non-caucasians. This should be clarified.
Moreover, it should be discussed that the apparent difference to RES response according to race could maybe be explained alone by a far higher standard deviation at least in the GTT and AUC in the non-caucasians compared to the Caucasians. The higher SD must be the reason why differences in GTT and AUC do not reach significance within non-caucasian comparisons, while an almost similar percentage difference between RES/placebo reach highly significant p values within the Caucasian subgroup. And could there be any explanation to why SDs are so much higher in the non-caucasian group? Which races are represented? Several different races pooled to an "non-caucasian" group?

3)
In the section "Power Analysis and Sample Size" the authors state that "…a sample size of 45 subjects in each group provides balance for comparison of study outcomes…", but include only 14 in each group (due to low variability in clamps which is the primary outcome). Could the the authors elaborate on this? The risk of type 2 error at least on secondary outcomes is substantial, and should be mentioned in the discussion. Also, in section 3.3 results should not be mentioned, only the description of the methods used.

4)
Page 6, line 19-24: "To our knowledge, this is the first human study to determine the effects of the RES on obese men with the MetS, under stable metabolic conditions in a randomized, placebo controlled trial."
In 2017 Kjær et al. published a human RCT study on the effects of RES on obese men with MetS. They were kept weight neutral and with no exercise habit changes during the 16 week study. The metabolic condition may not have been as stable as during this submitted work, however, the results from Kjær et al should be mentioned and discussed in this paper due to similarity of studies, and to some extent comparable findings (https://doi.org/10.1210/jc.2016-2160).
This submitted paper mentions several papers that report positive effects of RES on e.g. glucose metabolism (more or less clinically relevant). In addition to the above mentioned paper, others could also be mentioned briefly to reflect the very ambiguous in human trials (e.g. doi: 10.3945/ajcn.115.117440; doi: 10.2337/db12-0975; doi: 10.1016/j.cmet.2012.09.015).

5)
Why only 10+11 samples of adipose tissue?

Minor comments:
Page 4, line 21: MetS is not a disease, it is rather a cluster of conditions.
Figure 1 in the "Allocated to placebo" -box: the last 1,5 line should be deleted?
Figure 2: colors in the middle upper panel are difficult to discriminate. Expand the legends to increase the readers understanding
Please describe in detail both Figures and Tables in the legends

Authors' rebuttal

REVIEWER comment	AUTHORS' response ADRESSED	NOTES
Walker et al. submitted an interesting manuscript on the effect of resveratrol on obese men with metabolic syndrome, and the features associated with MetS. RES has been investigated extensively in pre-clinical settings, and has been suggested to positively impact several biological systems, including glucose metabolism. This study seems well planned and very comprehensive, including clamp, glucose tolerance test, resting energy expenditure, adipose tissue biopsy, and fecal microbiota. The authors report only a marginally altered glucose homeostasis overall, however, in subanalyses, this seems to be driven by a more convincing effect within a very small group of Caucasians, while non-caucasians had no effect. Fecal microbiota were also affected differently according to race. Other features of the MetS were not affected by RSV.	Thank you	
1) It is unclear if the study participants were "admitted" 24-hours/day at the metabolic unit during the entire 35 day study? This would provide extremely controlled conditions, which overall is good, but will certainly affect comparisons intra-individually pre- and post-	Subjects slept in the hospital, took breakfast and dinner in the hospital, and where permitted to go out on pass during the day with a packed lunch. They were instructed to only eat provided food.	Pages 10, lines 1-3

treatment. The randomized design will of course make up for this.		
2) The differences between the Caucasians and noncaucasians in relation to response to RES on glucose homeostasis are described (within the results section) as differences comparing the races: "As shown in Table 4B, there were highly significant effects on insulin resistance as measured by the insulin clamp technique in	These have been clarified See tables 4a and 4b for comments	Page 18, lines 21-22 Page 18, lines 2-3 Page 40, line 19 Page 41, lines 7-9

Caucasian when compared to non-Caucasian subjects ($p<0.001$). Furthermore, the 120 minute end point glucose level and the glucose concentration area under the curve during the glucose tolerance test were significantly lower in Caucasian subjects (Table 4B). At baseline, these two groups did not differ in age, BMI or the number of components of the metabolic syndrome (Data not shown)." The tables showing the data (table 4a and 4b) instead reveal comparisons between the treatment groups (RES vs placebo) when divided in Caucasians and non-caucasians. This should be clarified. Moreover, it should be discussed that the apparent difference to RES response according to race could maybe be explained alone by a far higher standard deviation at least in the GTT and AUC in the non-caucasians compared to the Caucasians. The higher SD must be the reason why differences in GTT and AUC do not reach significance within non-caucasian comparisons, while an almost similar percentage difference between RES/placebo reach highly significant p values within the Caucasian subgroup. And could there be any explanation to why SDs are so much higher in the non-caucasian group? Which races are represented? Several different races pooled to an "non-caucasian" group?		

Es 7-3) In the section "Power Analysis and Sample Size" the authors state that "…a sample size of 45 subjects in each group provides balance for comparison of study outcomes…", but include only 14 in each group (due to low variability in clamps which is the primary outcome). Could the the authors elaborate on this? The risk of type 2 error at least on secondary outcomes is substantial, and should be mentioned in the	Sample size corrected to 14 in each group. This was a typo Dispersion of non-Caucasian's 120 minute glucose concentration could decrease the likelihood of significant findings in this racial group. The potential for type 2 error is discussed. The study was powered for the primary endpoint of	Page 15, line 21 Page 16, line 4 Page 23, lines 19-21 Page 24, lines 3-5
discussion. Also, in section 3.3 results should not be mentioned, only the description of the methods used.	insulin resistance, posing the potential for a type 2 error in analysis of the secondary outcomes. Section 3.3, results statement moved to results section	 Page 16, lines 14-16 moved to page 18, lines 21-22

4) Page 6, line 19-24: "To our knowledge, this is the first human study to determine the effects of the RES on obese men with the MetS, under stable metabolic conditions in a randomized, placebo controlled trial." In 2017 Kjær et al. published a human RCT study on the effects of RES on obese men with MetS. They were kept weight neutral and with no exercise habit changes during the 16 week study. The metabolic condition may not have been as stable as during this submitted work, however, the results from Kjær et al should be mentioned and discussed in this paper due to similarity of studies, and to some extent comparable findings (https://doi.org/10.1210/jc.2016-2160). This submitted paper mentions several papers that report positive effects of RES on e.g. glucose metabolism (more or less clinically relevant). In addition to the above mentioned paper, others could also be mentioned briefly to reflect the very ambiguous in human trials (e.g. doi: 10.3945/ajcn.115.117440; doi: 10.2337/db120975; doi: 10.1016/j.cmet.2012.09.015).	This study was tightly controlled, with common diet, weight stabilization, monitoring of activity, and administration of the randomized drug by RNs. Other studies did not control the metabolic conditions of diet, weight and activity, RES/placebo doses were self-administered, and the studies were conducted in the community. We appreciate the advice to include other studies in relation to our findings, and have done so in the discussion section of our paper.	Page 22, lines 13-23 Page 23, lines 1-6 References: Page 31, lines 19-22 Page 32, lines 12-16 Page 33, lines 18-22 Page 34, lines 16-20
5) Why only 10+11 samples of adipose tissue?	Additional consent was required for RNA sequencing. 21 consented, 6 did not give consent, and one subject did not undergo biopsy due to anxiety	Page 13, lines 13-15

Minor comments: Page 4, line 21: MetS is not a disease, it is rather a cluster of conditions. Figure 1 in the "Allocated to placebo" -box: the last 1,5 line should be deleted? Figure 2: colors in the middle upper panel are difficult to discriminate. Expand the legends to	Disease changed to condition Yes, corrected (deleted) Colors changed to improve discrimination Legend amended to reflect change in color coding Additional language reflecting the results	Page 4, line 11 Page 44 Page 47, figure 2, lines 9-10 Page 47, lines 11-13
increase the readers understanding Please describe in detail both Figures and Tables in the legend		
Reviewer #1: Dear Authors, Thank you for allowing me to review this manuscript, which lays down further information about the potential role of resveratrol as a therapy for treating the metabolic syndrome by presenting the results of a pilot RCT. I have specific comments regarding sections of the text below.	Thank you	
ABSTRACT Please refer to "coronary heart disease" or "coronary artery disease" rather than "coronary disease"	Changes made as suggested. Coronary disease is changed to coronary heart disease.	Page 2,line 4 Page 6, line 3

After "The polyphenol resveratrol (RES) is believed to improve glucose homeostasis and insulin resistance by activating sirtuin, which acetylates and co-activates downstream targets." please add a few words to summarize how acetylating and co-activating downstream targets improve glucose homeostasis	Sirt 1 affects glucose and lipid homeostasis in the liver, insulin secretion in the pancreas, and glucose uptake in skeletal muscle.	Page 2, lines 6-8
RELEVANCE FOR PATIENTS This is a pilot study and while the significant changes seen in the small sub-group offer support to pursue research in this direction, they are not sufficient to support the use of resveratrol in patients with the metabolic syndrome. In addition, the cost of such a large dose of resveratrol is not necessarily low nor costeffective given that the cost of first-line diabetes medication such as metformin is also low and has been shown to have a much greater effect on glucose homeostasis in multiple large-scale clinical trials that what this study has shown. Please modify this section to reflect the degree of uncertainty in applying these results to patients, and remove reference to the low cost of	Low cost wording removed In the US, metformin is a prescription drug and would not be routinely available to people with pre-diabetes or MetS. The cost of metformin is very variable depending on insurance status of patient and type of insurance, as well as the cost of doctor visit for prescription. Reference to the Timmers studies that showed similar results in their population which was exclusively Caucasian.	Page 3. Line 11 Page 3, lines 12-13
resveratrol.		

INTRODUCTION Page 4, line 45: This sentence should be revised. Resveratrol is more commonly extracted from the Japanese knotweed plant for dietary supplements. Grapevine leaves also contain significant amounts of resveratrol and may be a better source for extracts as they are produced in large amounts as waste products from the grape growing industry. Other natural sources also include certain berries and peanuts.	The trans-resveratrol used in the study was exclusively organic Japanese knotweed. Japanese knotweed with reference (Chen) and grapes added to details of RES	Page 9, lines 13-14 Page 4, lines 21-22 Page 29,lines 20-21 Page 30,lines 1-2
Page 4, line 51: The Zamora reference is not provided in the reference list	Zamora reference added.	Page 36, lines 1-4
Page 5, line 41-43: resveratrol is modified by the gut microbiota primarily in the large intestine, and not the stomach. Please revise use of "gastrointestinal tract"	Used term colon instead of gastrointestinal track	Page 5, line 20
Page 5, line 48-51: Please describe how resveratrol affects TMAO production and its relevance for MetS.	Effects of RES on TMAO production expanded, with reference to decrease in development of atherosclerosis and heart disease (co-morbidities of MetS.)	Page 6, lines 1-3

MATERIALS AND METHODS Page 7, line 46-51: The description of screening results is different to what is reported in the Results section. Perhaps this paragraph can be taken out and any extra information added to the repeated information in the results section? In any case, the clamp procedure was listed as an inclusion criteria, yet subjects appear to have been included before this procedure had taken place. Perhaps it was a secondary screening procedure? Or a baseline procedure that was not part of the inclusion criteria? Page 7, line 58: It would be helpful to describe the race/ethnicity of the non-Caucasians if possible, especially given differences in diet and risk of diabetes in various ethnic groups that may contribute to null results in non-Caucasians. Page 8, line 57-60: Please describe the type of	The use of the baseline clamp as the final screening for this study is explained. Since the clamp is very time consuming and could be influenced by diet and activity, it was performed while the subjects were in-house on a Western style diet for 4 days. It was the first baseline test done so that, if the subject was insulin sensitive, he would not have undergone any other testing. Subjects were aware that they may be withdrawn from the study if they were insulin sensitive, and consented to this in the informed consent. We have edited the text to reflect this. Race/ethnicity added for non-Caucasians RES from Japanese knotweed.	Page 7, line 12 Page 8, lines 2-5 Page 8, lines 11-12 Page 9, lines 13-14

resveratrol in the product and its source. Page 9, line 26-29: Please state whether subjects' weight was including clothing Page 10, line 36-40: Please state when the fecal samples were obtained. Often the sampling occurs at home and the samples are brought in frozen to the center, but it is possible that fecal samples were taken during the study. Please clarify. Page 15, line 4-7: the sample size calculation indicated that 45 subjects in each group were required, yet only 14 were finally included.	Subjects were weighed in a hospital gown after an overnight fast and post voiding Fecal samples were obtained in the hospital and immediately frozen. The sample should have been 14 in each group. This was a typo and has been corrected.	Page 10, lines 9-10 Page 11, line 13-14 Page 15, line 21 Page 16, line 4

Please comment on the effect of the final sample size on the relevance of the results.		
Additional comment: please consider adding a description of how the subjects were randomized into the study, and how the investigational product was kept double-blinded to receive the maximum score on the Jadad scale (Jadad et al., 1996).	Randomization was done by the research pharmacist using a web based randomization program. The study team and subjects were blinded for the entire study to avoid bias. The pharmacist assigned the group when subjects met inclusion criteria after baseline clamp. RES and placebo were made in identical capsules by Candlewood Stars. The research pharmacist dispensed the drug to the patient med box in the locked medication room in the hospital, where it was administer by professional nurses who were blinded.	Page 8, lines 21-23 Page 9, lines 17-19

RESULTS Page 17, line 10-15: The description of the flow of subjects through the study does not match up with the flow chart in figure 1. The description states that 14 subjects were randomized into each group, however the flow chart indicates that 17 and 16 were randomized, and there were 5 drop outs. The description claims that there were 34 eligible subjects, but figure 1 states that 33 were eligible. As insulin resistance was considered to be an inclusion criterion, I would normally assume that the clamp procedure was performed before randomization, however the way that it is	Description corrected to read 31 eligible, three withdrawn. The flow chart has been corrected to indicate that 31 subjects were eligible	Page 18, lines 5-6 Page 8, lines 5-9 Page 44 Flow chart, lines 14,16,17 Page 45,lines 1,2,4
described and appears in the flow chart indicates that the clamp was performed after randomization. Please correct, or address this departure from clinical trial norms.		

DISCUSSION Page 21, line 19: The results are inconclusive, not negative. Page 21, lines 51-54: Bode does not investigate the effect of race, therefore state explicitly here that the results could be a chance finding due to high inter-person variability in RES absorption or metabolism. An additional point to make here is that there may be more heterogeneity in diets in the non-Caucasian group (presumably consisting of different race-ethnicity groups such as African Americans and Asians, who have distinctive dietary patterns), leading to greater variability in measures of glucose homeostasis in the nonCaucasian group. Page 23, line 9: These were null findings, not negative findings.	Negative changed to inconclusive In the manuscript we do not indicate that Bode investigated the effect of race. Our subjects ate the same diet throughout the 35 day hospitalization. The RES levels were drawn after 30 days on a fixed diet receiving RES/placebo BID. Negative findings changed to null	Page 22, line 13 Page 25, line 6
Clarification of Tables	Additional explanation of Tables added	Table 1, page 37, lines 11-12 Table 2, page 38, lines 19-20 Table 3A, 3B, page 40, lines 4-5 Table 4A,4B, page 41, lines 7-9 Table 5, page 42, lines 1-2 Table 7, page 44, line 2

2nd editorial decision

Date: 01-Nov-2018

Ref.: Ms. No. JCTRes-D-18-00018R1
The Effects of Trans-Resveratrol on Insulin Resistance, Inflammation, and Microbiota in Men with the Metabolic Syndrome A Pilot Randomized, Placebo Controlled Clinical Trial
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Dear authors,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Comments from the editors and reviewers:

Reviewer #1: Dear Authors,

Thank you for considering my suggestions for improvements and incorporating them into the manuscript. I have no further comments or remarks.

Reviewer #2: Questions and comments are answered in a satisfactory manner.