PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	The effect of resveratrol supplementation on the expression levels of factors associated with cellular senescence and sCD163/sTWEAK ratio in patients with type 2 diabetes mellitus: study protocol for a double-blind controlled randomized clinical trial
AUTHORS	Abdollahi, Shima; Salehi-Abargouei, Amin; tabatabaie, Mahtab; sheikhha, Mohammad Hasan; Fallahzadeh, Hossein; Rahmanian, Masoud; Toupchian, Omid; Karimi-Nazari, Elham; mozaffari-khosravi, hassan

VERSION 1 - REVIEW

REVIEWER	Inar Castro University of Sao Paulo - Brazil
REVIEW RETURNED	08-Oct-2018

GENERAL COMMENTS	This is a study protocol and not a manuscript. Thus, my recommendation "accepted" is just applied to the protocol. It is an interesting study in which the authors will investigate the effect of trans-resveratrol supplementation on biomarkers of atherosclerosis associated to diabetes type 2. The hypothesis seems to be well supported by the literature and methods are adequate, including statistical analysis. Besides the effect of the intervention on intima hyperplasia, the study will evaluate some suggested mechanisms involved in the results. Ethics aspects are being attended. I suggest the authors are very careful about the individual response of the patients.

REVIEWER	Dr. Marlies de Ligt Maastricht University, The Netherlands
REVIEW RETURNED	17-Jan-2019

GENERAL COMMENTS	BMJ Open: Review report “The effect of resveratrol supplementation on the expression levels of factors associated with cellular senescence and sCD163/sTWEAK ratio in Patients with Type 2 Diabetes Mellitus: Study protocol for a double blind Controlled Randomized Clinical Trial”

	In the submitted study protocol, a study set-up is described for examining the effects of resveratrol supplementation in T2D patients on cellular factors associated with intima hyperplasia through cellular pathways. In relation to this specific topic no human clinical trial has been performed so far, which makes this an interesting study. However, I have some comments that need to be addressed to improve the quality of the study protocol. Major comments The abstract misses some essential information. A sentence should be added linking vascular function to the chosen pathways of interest. Furthermore, in the methods abstract section should be mentioned that also clinical outcome parameters will be measured (e.g. serum cholesterol). Finally, the words 'parallel and placebo' should be added to the description of type of trial: randomized parallel placebo-controlled clinical trial). The strengths and limitations section is too limited. No real so-to-say functional outcome measures will be performed in this study. For example: no OGTT or hyper-insulinemic clamp is performed and more importantly vascular function is not measured by FMD, PAT etc. Under Eligibility criteria it would be useful to add information about the type of medication that will be allowed for treating T2D. For example, are also patients allowed who are controlled by diet only? Furthermore, I was wondering if menstruation phase is considered when performing the measurements since fertile women will participate in the study. In continuation: are women on hormonal birth control allowed? Are the authors also planning to make a division in the data-analyses for separating pre and post-menopausal women and does the study have enough power for performing such a sub-analysis? The methods for describing Gene expression assay is too limited and should be extended. For example, the authors should provide detailed information on: which primers will be used (primer sequence), device to be used, annealing temperature, supplier of primers etc. Minor comments In the methods section again the word 'parallel' is missing in the description of the design. In the Eligibility criteria section it's unclear to me whether the participants are not allowed to have drunk red wine in the past 6 months, or whether they are not allowed to have consumed food supplements containing red wine for the past 6 months. This should be clarified. Under Intervention it should be clarified which questionnaires are used. Now it just states: 'related questionnaires.' The protocol contains some awkward sentences. I advise the authors to re-read the protocol. For example: page 9 the last sentence is unclear.

REVIEWER	Rinkoo Dalan Tan Tock Seng Hospital, Singapore
REVIEW RETURNED	18-Feb-2019

GENERAL COMMENTS	This is a well written clinical trial protocol for publication. I have some questions and concerns. 1. English language editing needs to be done for eg> a. Line 103 - the sentence starts with uncontrolled DM without a capital letter. b. line 105: it should be: Atherosclerosis is one of the most important cause of CVD.. actually better written as Intima hyperplasia is a cardinal manifestation of atherosclerosis which leads to CVD. line 210: spelling mistake -previous Under gene expression: PBMCs should be peripheral blood mononuclear cells - its written as nuclear cells 2. Why was the duration of treatment or supplementation chosen as 8 weeks. Is 8 weeks sufficient time to see the changes in primary outcome? 3. Limitations of the study in terms of study design is not mentioned in the clinical trial. Especially since bottles are labelled as A, B a possible limitation is that from adverse effects reported or when unblinding is done for any reason , the entire group will be unblinded. It is usually recommended to use serial number on the bottles. Since the assay results are objective and unlikely going to be affected by this it should be alright but should be stated as a limitation. They have not stated as to how allocatiion will be done and kept blinded. Who will do randomisation, allocation and then prescription to patient ? Other strengths and limitations need to be elaborated upon as well.

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author
Reviewer: 1
Reviewer Name: Inar Castro
Institution and Country: University of Sao Paulo - Brazil
Please state any competing interests or state ‘None declared’: None declared
This is a study protocol and not a manuscript. Thus, my recommendation "accepted" is just applied to the protocol.
It is an interesting study in which the authors will investigate the effect of trans-resveratrol supplementation on biomarkers of atherosclerosis associated to diabetes type 2. The hypothesis seems to be well supported by the literature and methods are adequate, including statistical analysis. Besides the effect of the intervention on intima hyperplasia, the study will evaluate some suggested mechanisms involved in the results. Ethics aspects are being attended. I suggest the authors are very careful about the individual response of the patients.

We would like to express our appreciation for your attention and your thorough understanding for need such as these studies. Thanks for spending the time to read this paper and found it interesting. Revision has been made according to the suggestions. We will consider individual

responses in complementary analyses based on participant's characteristic as far as possible (Please find these modifications on Page 15, line 358).

Reviewer: 2
Reviewer Name: Dr. Marlies de Ligt
Institution and Country: Maastricht University, The Netherlands
Please state any competing interests or state 'None declared': None declared
In the submitted study protocol, a study set-up is described for examining the effects of resveratrol supplementation in T2D patients on cellular factors associated with intima hyperplasia through cellular pathways. In relation to this specific topic no human clinical trial has been performed so far, which makes this an interesting study. However, I have some comments that need to be addressed to improve the quality of the study protocol which can be found in the attachment.

We would like to thank you for your attention and insightful comments. They gave us clear guidance and some positive critiques.

Reviewer: 3
Reviewer Name: Rinkoo Dalan
Institution and Country: Tan Tock Seng Hospital, Singapore
Please state any competing interests or state 'None declared': None declared
This is a well written clinical trial protocol for publication.
I have some questions and concerns.

1. English language editing needs to be done for eg>
a. Line 103 - the sentence starts with uncontrolled DM without a capital letter.
b. line 105: it should be: Atherosclerosis is one of the most important cause of CVD.
actually better written as Intima hyperplasia is a cardinal manifestation of atherosclerosis which leads to CVD.
line 210: spelling mistake –previous Under gene expression:
PBMCs should be peripheral blood mononuclear cells - its written as nuclear cells
Thank you, based on your comments all spelling, grammatical sentences, and mistake expression were corrected by a native English speaking editor and the certificate has been uploaded.

2. Why was the duration of treatment or supplementation chosen as 8 weeks. Is 8 weeks sufficient time to see the changes in primary outcome?
Thank you for pointing this out. We reviewed studies on resveratrol supplementation in diabetic patients and concluded that the highest well-tolerable intervention duration is 8 weeks. Moreover, the supplement used in our study is pure micronized-resveratrol with more bioavailability which can increase the likelihood of digestive problems in long duration supplementation. To our knowledge, there is no human study to assess the effect of resveratrol supplementation on our interesting genes. However, in studies evaluated the endothelial function, resveratrol showed a significant beneficial effect even with a single dose (1) or 6 weeks administration (2).

1. Marques BC, Trindade M, Aquino JC, Cunha AR, Gismondi RO, Neves MF, Oigman W. Beneficial effects of acute trans-resveratrol supplementation in treated hypertensive patients with endothelial dysfunction. Clinical and Experimental Hypertension. 2018 Apr 3;40(3):218-23.

2. Wong RH, Berry NM, Coates AM, Buckley JD, Bryan J, Kunz I, Howe PR. Chronic resveratrol consumption improves brachial flow-mediated dilatation in healthy obese adults. Journal of hypertension. 2013 Sep 1;31(9):1819-27.

3. Limitations of the study in terms of study design is not mentioned in the clinical trial. Especially since bottles are labelled as A, B a possible limitation is that from adverse effects reported or when unblinding is done for any reason, the entire group will be unblinded. It is usually recommended to use serial number on the bottles.
Since the assay results are objective and unlikely going to be affected by this it should be alright but should be stated as a limitation.
Other strengths and limitations need to be elaborated upon as well.
Thank you for this interesting insight. We have now added this under the Strengths and Limitations section (Please find this information on Page 15, lines 371-383).
They have not stated as to how allocation will be done and kept blinded. Who will do randomization, allocation and then prescription to patient?
Thank you for your suggestion. We have now provided these details under the section Randomization (Please find this information on Page 8, lines 186-196).

VERSION 2 – REVIEW

REVIEWER	Rinkoo Dalan Tan Tock Seng Hospital Singapore
REVIEW RETURNED	10-Apr-2019

GENERAL COMMENTS	The authors have made a revision addressing the comments.