PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Actions of annatto-extracted tocotrienol supplementation on obese postmenopausal women: study protocol for a double-blinded placebo-controlled randomized trial
AUTHORS	Aryaie, Amir; Tinsley, Grant; Lee, Jaehoon; Watkins, Bruce; Moore, Lane; Alhaj-Saleh, Adel; Shankar, Kartik; Wood, Sarah; Wang, Rui; Shen, Chwan-Li

VERSION 1 – REVIEW

REVIEWER	Oran Kwon Ewha Womans University, Republic of Korea
REVIEW RETURNED	06-Oct-2019

GENERAL COMMENTS	Bmjopen-2019-034338 The manuscript is well-founded and relatively easy to follow. Therefore, the reviewer has only minor points below that, if addressed, may further strengthen the manuscript. • Abbreviations should be defined at first mention and used consistently thereafter, e.g., HETE on page 3 line 39; GRAS on page 9 line 39; and VAT on page 11 line 53. • Provide the approval number of Texas Tech University Health Sciences Center IRB at page 7. • One of the exclusion criteria of this study includes “changes in medications/supplements that could affect lipid metabolism.” This statement does not seem to match with the randomization and allocation concealment section, which describes that the subjects will be stratified according to the use of medications affecting lipid metabolisms like hormone replacement therapy and

	cholesterol-lowering drug. Please clarify the exclusion criteria or the randomization and allocation concealment to avoid this contradiction. • Please, describe more about the chemical characteristics of test material, annatto tocotrienols. • Adipose tissue will be used to analyze gene expression, OxL and eCB. However, the sample collection section at page 10 doesn't describe how the adipose tissue will be collected.

REVIEWER	Magdalena Sobieska Karol Marcinkowski University of Medical Sciences, Poznan, Poland
REVIEW RETURNED	29-Oct-2019

GENERAL COMMENTS	The decision to use annatto-derived TT should be described in more details.

REVIEWER	Rafaella Luna UFPI - Brazil
REVIEW RETURNED	22-Nov-2019

GENERAL COMMENTS	The aim of the study is to assess the safety and efficacy of TT consumption for lipid-related parameters in obese postmenopausal women. This a important study integrating various tools such as genomics, metabolomics and metagenomics. This study will guide the future efficacy TT interventions and TT can be implemented as an alternative for obese population in anti-obesity management. I have some considerations for the authors: Title The title mentions annatto-extracted tocotrienol supplementation. However, annatto is not mentioned in the study methodology. Methodology Sample collection – Where will blood samples be taken: home, hospital? Page 11, line 1: "We will measure adherence to the intervention by counting the softgels consumed and determine compliance by the percentage of all softgels ingested" – question: Will some percentage be considered for exclusion from the study? Page 11, line 3: "...also measure serum concentrations of TT and tocopherol at baseline and 24-week visit as additional evidence for adherence and compliance." – question: Will some value be considered to adherence to the study?

VERSION 1 – AUTHOR RESPONSE

Reviewer #1 (Rafaella).
Title
"The title mentions annatto-extracted tocotrienol supplementation. However, annatto is not mentioned in the study methodology.
Response: we have mentioned annatto- in this methodology.

Methodology
"Sample collection – Where will blood samples be taken: home, hospital?"
Response: The blood samples will be taken at Clinical Research Institute, TTUHSC. We have included this venue in the R1, Method section.

"Page 11, line 1: "We will measure adherence to the intervention by counting the softgels consumed and determine compliance by the percentage of all softgels ingested" – question: Will some percentage be considered for exclusion from the study?"
Response: No, we will not use the percentage of softgels consumed as our exclusion criteria. We have included such info in this R1, Method section.

"Page 11, line 3: "...also measure serum concentrations of TT and tocopherol at baseline and 24-week visit as additional evidence for adherence and compliance." – question: Will some value be considered to adherence to the study?"
Response: Yes, the serum concentration of TT and tocopherol are considered as a form of adherence to the study and we will not use such data as our exclusion from the study. We have included such info in this R1, Method section.

FORMATTING AMENDMENTS (if any)
"Required amendments will be listed here; please include these changes in your revised version:
● Please write a correct format of abstract for protocol paper. Below is your guide:

Introduction, Methods and Analysis, Ethics and Dissemination"
Response: In this R1, we have formed our abstract as shown in guideline accordingly.

Reviewer #2
Bmjopen-2019-034338
"The manuscript is well-founded and relatively easy to follow. Therefore, the reviewer has only minor points below that, if addressed, may further strengthen the manuscript."
Response: Thank you.

"Abbreviations should be defined at first mention and used consistently thereafter, e.g., HETE on page 3 line 39; GRAS on page 9 line 39; and VAT on page 11 line 53."
Response: In this R1, we have defined abbreviations at first mentioned throughout the text accordingly.

"Provide the approval number of Texas Tech University Health Sciences Center IRB at page 7."
Response: IRB approval number has been provided accordingly.

"One of the exclusion criteria of this study includes "changes in medications/supplements that could affect lipid metabolism." This statement does not seem to match with the randomization and allocation concealment section, which describes that the subjects will be stratified according to the use of medications affecting lipid metabolisms like hormone replacement therapy and cholesterol lowering

drug. Please clarify the exclusion criteria or the randomization and allocation concealment to avoid this contradiction.
Response: Thank you for comment. I have clarified this – “changes in medications/supplements that could affect lipid metabolism” has been removed from exclusion criteria. Instead, the following item has been included in the exclusion criteria.
“Changes to medications or supplements (i.e., steroids, statins) within 3 months of the baseline study visit that could affect lipid metabolism. • If they change any medications/supplements after the baseline visit that will affect lipid metabolism, their study participation will end.”

“Please, describe more about the chemical characteristics of test material, annatto tocotrienols.”
Response: In this R1, we have provided more about the chemical characteristics of annatto tocotrienols in Method section.

“Adipose tissue will be used to analyze gene expression, OxL and eCB. However, the sample collection section at page 10 doesn’t describe how the adipose tissue will be collected.”
Response: In this R1, we have included info how the adipose tissue will be collected.

VERSION 2 – REVIEW

REVIEWER	Oran Kwon Ewha Womans University, Republic of Korea
REVIEW RETURNED	15-Dec-2019

GENERAL COMMENTS	No further comment.

REVIEWER	Rafaella Cristhine Pordeus Luna Federal University of Piaui
REVIEW RETURNED	08-Dec-2019

GENERAL COMMENTS	The authors answered and completed the information for a better understanding of the article.