PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Dietary determinants of serum total cholesterol among middle-aged and older adults: a population-based cross-sectional study in Dar es Salaam, Tanzania
AUTHORS	Kakarmath, Sujay; Zack, Rachel; Leyna, Germana; Fahimi, Saman; Liu, Enju; Fawzi, Wafaie; Lukmanji, Zohra; Killewo, Japhet; Sacks, Frank; Danaei, Goodarz

VERSION 1 - REVIEW

REVIEWER	Erkki Vartiainen National Institute for Health and Welfare Finland
REVIEW RETURNED	11-Nov-2016

GENERAL COMMENTS	General: This is an important paper. Increasing use of palm oil in developing countries is likely to increase cardiovascular mortality in near future. Paper is well written, statistical analyses are correctly done and the main messages are clear. Specific: In methods section BMI do not have underweight but it is reported in table. In table 2 footnote b: Total caloric is not reported in table only fat intake in grams. Usually when you are adjusting by confounding factors the association is decreasing. Here the difference between palm oil and other oil uses increase from 14 mg/dL to 17 mg/dL and the effect of meat consumption increased from nonsignificant (p 0.550) 3 mg/dL to significant 16 mg/dL (p 0.026). You may add in the discussion which of the co-variates is causing these effects. In the discussion section you may add price difference between the oils. It looks like lower socioeconomic groups are using more palm oil. This may increase the CVD mortality differences between the socioeconomic groups in the future. Is there any information of the CVD mortality trend or differences between the socioeconomic groups?

REVIEWER	Rob M. van Dam Saw Swee Hock School of Public Health, National University of Singapore, Singapore
REVIEW RETURNED	16-Nov-2016

GENERAL COMMENTS	This is a cross-sectional study of socio-demographic and dietary

determinants of serum cholesterol levels in 356 urban residents of Tanzania. Among other findings, the type of cooking oil used is associated with serum cholesterol levels suggesting that replacement of palm oil with non-tropical vegetable oils may be beneficial. This is of interest as data on palm oil use and serum cholesterol levels from population-based studies is limited. My comments are shown below.

1). No distinction between cooking oils other than palm oil was made in the analysis relating oil use to serum cholesterol concentrations. It will be useful to include a direct comparison of palm oil with sunflower oil as such a specific comparison will be easier to interpret meaningfully.

2). For the dietary analyses, the authors should conduct a sensitivity analysis excluding those who have been told they have hypercholesterolenemia as reverse causation may have occurred (they may have been advised to change their diet).

3). It would be very desirable to provide more information on the key measurements used. What was the CV% for the serum cholesterol measurements? Is there any data on the validity or reproducibility of the FFQ? What exact question was asked about cooking oil use?

4). The results on dietary determinants of serum cholesterol concentrations would be more comprehensive if results on intake of fatty acids (% of energy intake) as determinants of serum cholesterol would be reported.

Specific comments:
5). Introduction: The Introduction only refers to previous studies on dietary determinants of serum cholesterol in Tanzania, but it will be useful to take a broader perspective mentioning data from there countries in the region. In addition, given the focus on cooking oils it will be of interest to mention previous data on cooking oils and serum cholesterol levels.

6). Table 1: numbers are missing for using nuts and legumes >1 serving per day for 'other cooking oil' users.

7). Table 1: given the substantial difference is total energy intake by cooking oil use, it will be better to express fat intakes as % of total energy intake rather than grams.

REVIEWER	Federica Laguzzi Karolinska Institutet, Institut of Environmental Medicine, Unit of Cardiovascular Epidemiology, Sweden
REVIEW RETURNED	29-Nov-2016

| GENERAL COMMENTS | Interesting manuscript reflecting a need in this field to understand better the association between certain food intake trend (as palm oil) and an increased prevalence of metabolic and chronic disease as CVD in developing countries. The main finding of this study (palm oil) may also add knowledge useful for future nutritional recommendation to decrease total cholesterol in this population. However, there are several redundancy and weak points including lack of novel aspect in the research question (dietary factors in relation to serum total cholesterol have been extensively studied), |

few numbers of participants, not clear explanation of methods as well not clear presentation of results.

Here suggestions and comments on the manuscript:

TITLE
Line 6: I would suggest to rephrase the title in Dietary determinants of serum total cholesterol among middle aged and older adults: cross sectional results in a population based in Dar es Salaam, Tanzania or to use cross-sectional population based study

ABSTRACT:
I think that it is not accurate:
Line 11: I suggest to indicate that participants were both men and women
Line 14-15: I suggest point-of-care
Line 19-20: demographic might include socioeconomic factors. I think it is redundant.
Line 25-26: It is better to be precise on what higher meat and lower meat indicate in your study.
Line 30: Please, check to be consistent in the use of tenses.
Line 31-32: As you reported, there are differences between Bantu and other ethnic groups in Tanzania as well among those who are living in pastoral, rural or urban city. I don't think (based also on your introduction) you can really generalize these results to the entire Tanzania.

INTRODUCTION
Line 20-22: I think it is a result of Ref 5 and not 4.
Line 29-31: I suggest to add a sentence why it is important to study the relation between dietary factors in relation to serum cholesterol. Maybe also a short description how the dietary habits differ by areas and how it is the traditional diet in Tanzania.
Line 31-35: See comment above, abstract, line 25-26.
Line 37-38: Since differences in the dietary habits it might be interesting to indicate in which areas (rural, urban, pastoral) these other studies were performed.
Line 41-45: I don`t think this sentence is relevant for the purpose of the study.
Line 47: dietary determinants instead of determinants.

METHODS:
It is comprehendible but not well organized and fully explained:

Line 18: it is a subsample from DUCS-HTN that it was selected for this study? Please make it clear. I think that the authors should also take in consideration how the selection of the area might have affected your results.

Measurements:
To make it clear I suggest to divide these paragraph in exposures, serum total cholesterol and confounders measurements. Is the questionnaire validated? Since several dietary items were available, why have you only chosen certain group of food. It would be also interesting to see how other specific dietary intake of dairy or meat are related to serum cholesterol in this population. Moreover, the authors explained how the nutrients intake was calculated but I think it is more relevant for the purpose of the study to define how they treated the dietary variables used as well how question on use of oils was formulated. ¨

Page 8 Line 7: I would suggest to move all this paragraph on the statistical analysis since are covariates used to adjust crude relations between diet and serum cholesterol. Moreover, I suggest to state clearly which of these factors were used as covariates. About the wealth index, please explain the reason to use a statistical method of difficult interpretation to create a covariate. Then, please give description how PC were retained

Page 8 line 36-39: you describe your exclusion factors in the results section. Please move it there.
Page 8 line 41: the author use linear regression to estimate the relation between diet and serum cholesterol. However, it really hard to understand which factors were used as exposure and which as covariates. Moreover, please explain how the adjustment for interviewers was done. Please rephrase the content of this paragraph.

RESULTS:

Results are not presented clearly. Sometimes tables and figures are not reflecting the aim of this study and it seems that the original aim of this project was to explore several factors related to serum cholesterol and not diet.

Table 1: I would suggest to drop the column oil palm. Since it is a results I would suggest to put it in another table after table 2. Moreover, I suggest to give more emphasis to serum cholesterol distribution (maybe histogram of the distribution)

Page 9 Line 21-41: it is not clear that all this paragraph is describing the table 1.

Table 2: This should be the table of results of the relation between diets and serum cholesterol. But title of the table does not report it. I suggest to have only results of the relation between diet and serum total cholesterol (crude and adjusted). Results are presented unclearly with exposure and covariates in the same table. Moreover, if possible I would suggest to categorize alcohol consumption (none, low, moderate and heavy). Still, I would suggest to have another table with the distribution (and possibly correlation) between several covariates/variables and total serum cholesterol.

Page 13: The author should comment also width of the confidence interval.

DISCUSSION

Several part are redundant

Page 16 Line 32-36: Please, explain why insufficient statistical power and measurements should be applicable only to fish intake?
Page 15 Line 40-41: this sentence is redundant with the sentence in line 32-34
Page 17 Line 5: I would add the small sample and how this might have affected the results.
Page 17 Line 45: I would make shorter your discussion on interventional program. And I would move it before the limitation and strength.
Page 18 Line 12: it should be your conclusion but it is not clear.

	Please make it shorter, and try to don t leave the main focus of your study.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Erkki Vartiainen

General:
This is an important paper. Increasing use of palm oil in developing countries is likely to increase cardiovascular mortality in near future.
Paper is well written, statistical analyses are correctly done and the main messages are clear.

Specific:
In methods section BMI do not have underweight but it is reported in table.
We have added underweight to BMI description in methods section (Page 7).

In table 2 footnote b: Total caloric is not reported in table only fat intake in grams.
We believe that this comment refers to Table 1 footnote b. Both caloric and fat intakes have been reported in Table 1. We have re-organized the table to put both total caloric intake close to fat intake.

Usually when you are adjusting by confounding factors the association is decreasing. Here the difference between palm oil and other oil uses increase from 14 mg/dL to 17 mg/dL and the effect of meat consumption increased from nonsignificant (p 0.550) 3 mg/dL to significant 16 mg/dL (p 0.026). You may add in the discussion which of the co-variates is causing these effects.
The direction of the change in an effect size due to confounding depends on the direction of the association between confounders and exposure and that of confounder and the outcome. In this case, confounding of the association of palm oil with serum total cholesterol is largely due to sex and meat intake. Female participants were less likely to report using palm oil and had higher serum total cholesterol. Similarly, those who consumed more meat were less likely to report using palm oil and had higher serum total cholesterol, therefore leading to negative confounding. We have added this observation to the Results (Page 14).

The association of meat intake with serum total cholesterol is (negatively) confounded by total energy intake. Higher meat consumption was associated with higher energy intake and higher energy intake was associated with lower serum total cholesterol. We have added this to the Results (Page 14).

In the discussion section you may add price difference between the oils. It looks like lower socioeconomic groups are using more palm oil. This may increase the CVD mortality differences between the socioeconomic groups in the future. Is there any information of the CVD mortality trend or differences between the socioeconomic groups?
We have added price difference in oils to the Discussion and mentioned their implication for future disparities in cardiovascular diseases (Pages 20,21).

Reviewer: 2
Reviewer Name: Rob M. van Dam
This is a cross-sectional study of socio-demographic and dietary determinants of serum cholesterol levels in 356 urban residents of Tanzania. Among other findings, the type of cooking oil used is associated with serum cholesterol levels suggesting that replacement of palm oil with non-tropical vegetable oils may be beneficial. This is of interest as data on palm oil use and serum cholesterol levels from population-based studies is limited. My comments are shown below.

1). No distinction between cooking oils other than palm oil was made in the analysis relating oil use to serum cholesterol concentrations. It will be useful to include a direct comparison of palm oil with sunflower oil as such a specific comparison will be easier to interpret meaningfully.

We have incorporated this suggestion in the revised manuscript. We excluded 9 participants who used 4 other types of cooking oil and reported the main results comparing palm oil with sunflower oil.

2). For the dietary analyses, the authors should conduct a sensitivity analysis excluding those who have been told they have hypercholesterolenemia as reverse causation may have occurred (they may have been advised to change their diet).

We have incorporated this suggestion as well. Table 2 now has an additional column where we excluded 16 participants who reported having hypercholesterolemia. The results and conclusions remain unchanged.

3). It would be very desirable to provide more information on the key measurements used. What was the CV% for the serum cholesterol measurements?

The within-person CV% for the CardioChek PA device has been estimated to be in the range of 1.3 to 2.9%. We have incorporated this information in the manuscript (Page 6). The between-person CV% in our study is 27.0%, similar to that of previous studies in Tanzania.[1]

1 Njelekela M, Negishi H, Nara Y, Tomohiro M, Kuga S, Noguchi T, Kanda T, Yamori M, Mashalla Y, Liu LJ, Mtabaji J. Cardiovascular risk factors in Tanzania: a revisit. Acta tropica. 2001 Jun 22;79(3):231-9.

Is there any data on the validity or reproducibility of the FFQ?

The FFQ was previously used in several studies in Tanzania. In the current study, we conducted both the FFQ and two 24-hour diet recalls. We are currently finalizing the analysis of comparing the FFQ with the diet recalls and our results indicate that the correlation between food items and nutrients in the FFQ with diet recalls is comparable to previous studies of nutritional intake in the developed and developing countries. For example, the table below shows the energy-adjusted and deattenuated rank correlation for the selected dietary factors used in the analysis which all fall within the acceptable range.

Item	Deattenuated rank correlation (95% CI)
Energy (kcal)	0.16 (0.02, 0.30)
Fat (% energy)	0.36 (0.19, 0.51)
Fruits and vegetables	0.03 (-0.17, 0.22)
Unprocessed red meat	0.47 (0.33, 0.59)
Fish	0.18 (-0.03, 0.38)
Dairy	0.35 (0.21, 0.48)

What exact question was asked about cooking oil use?

The exact wording was: "What is the kind of oil used most often in your home for frying food?". Frying is the most common cooking technique involving oil use in this population. The reference to a cooking technique was made to clearly distinguish it from other oils that may be used in a household, such as fuels. We have added this information to the Methods (Page 7).

4). The results on dietary determinants of serum cholesterol concentrations would be more comprehensive if results on intake of fatty acids (% of energy intake) as determinants of serum cholesterol would be reported.

We have intentionally avoided adding fatty acids to our regression model as a determinant of total cholesterol because fatty acid levels are affected by palm oil use and therefore it acts as a mediator for our main exposure of interest. Adjusting for such mediators in the regression model would

introduce bias due to collider stratification2.
2 Hernán MA, Hernández-Díaz S, Robins JM. A structural approach to selection bias. Epidemiology. 2004 Sep 1;15(5):615-25.

Specific comments:
5). Introduction: The Introduction only refers to previous studies on dietary determinants of serum cholesterol in Tanzania, but it will be useful to take a broader perspective mentioning data from there countries in the region.
We conducted a comprehensive literature search and revised the introduction to include data from other countries in the region (Pages 4, 5).

In addition, given the focus on cooking oils it will be of interest to mention previous data on cooking oils and serum cholesterol levels.
Additional paragraph added to introduction to include this information (Page 5).

6). Table 1: numbers are missing for using nuts and legumes >1 serving per day for 'other cooking oil' users.
Thank you for bringing this to our attention. We have made the necessary changes. We would like to point out that data from Tables seems to pick up errors AFTER the file has been uploaded. Prior to submission, we had fixed similar errors that we noticed in the preview on the submission website. The 'missing' numbers referred to above are not missing in the version of the document we have, and seem to be due to a problem with the file upload system.

7). Table 1: given the substantial difference is total energy intake by cooking oil use, it will be better to express fat intakes as % of total energy intake rather than grams.
Please see response to comment #4.

Reviewer: 3
Reviewer Name: Federica Laguzzi

Interesting manuscript reflecting a need in this field to understand better the association between certain food intake trend (as palm oil) and an increased prevalence of metabolic and chronic disease as CVD in developing countries. The main finding of this study (palm oil) may also add knowledge useful for future nutritional recommendation to decrease total cholesterol in this population.

However, there are several redundancy and weak points including lack of novel aspect in the research question (dietary factors in relation to serum total cholesterol have been extensively studied), few numbers of participants, not clear explanation of methods as well not clear presentation of results.

Here suggestions and comments on the manuscript:

TITLE
Line 6: I would suggest to rephrase the title in Dietary determinants of serum total cholesterol among middle aged and older adults: cross sectional results in a population based in Dar es Salaam, Tanzania or to use cross-sectional population based study
We have revised to move Dar es Salaam to the end.

ABSTRACT:
I think that it is not accurate:
Line 11: I suggest to indicate that participants were both men and women
We believe that the word 'adults' captures this information well.

Line 14-15: I suggest point-of-care
We have revised as suggested (Page 2).

Line 19-20: demographic might include socioeconomic factors. I think it is redundant.
Demographic and socioeconomic factors are distinct and are separately coded in online databases such as the Medical Subject Heading (MeSH) terms in MEDLINE. Therefore, we strongly prefer to keep them separate.

Line 25-26: It is better to be precise on what higher meat and lower meat indicate in your study.
We have revised as suggested.

Line 30: Please, check to be consistent in the use of tenses.
We have revised to make verb tenses consistent throughout.

Line 31-32: As you reported, there are differences between Bantu and other ethnic groups in Tanzania as well among those who are living in pastoral, rural or urban city. I don't think (based also on your introduction) you can really generalize these results to the entire Tanzania.
We have revised the Conclusion to clarify that results apply to this peri-urban population.

INTRODUCTION
Line 20-22: I think it is a result of Ref 5 and not 4.
We have corrected the reference.

Line 29-31: I suggest to add a sentence why it is important to study the relation between dietary factors in relation to serum cholesterol.
We have added a sentence to clarify the rational (Page 5).

Maybe also a short description how the dietary habits differ by areas and how it is the traditional diet in Tanzania.
Description of diet pattern in urban vs rural Tanzania has been added (Page 5).

Line 31-35: See comment above, abstract, line 25-26.
These studies measured food consumption in number of days per week and include them as continuous variables in the regression models. No thresholds have been defined for high or low consumption.

Line 37-38: Since differences in the dietary habits it might be interesting to indicate in which areas (rural, urban, pastoral) these other studies were performed.
We have added these details (Page 4).

Line 41-45: I don`t think this sentence is relevant for the purpose of the study.
It is not clear which sentence this comment refers to because the submitted manuscript did not include line numbers. We have edited the introduction to improve the relevance of all sentences to the study.

Line 47: dietary determinants instead of determinants.
We have removed this sentence.

METHODS:
It is comprehendible but not well organized and fully explained:

Line 18: it is a subsample from DUCS-HTN that it was selected for this study? Please make it clear.
We had clearly explained the sampling process for the current study and the parent DUCS-HTN study (Page 6).

I think that the authors should also take in consideration how the selection of the area might have affected your results.
This area was selected for the parent DUCS study because of the relative stability of its population which facilitates the work of the Demographic Surveillance Study. It is quite unlikely that such selection is jointly related to the participants use of palm oil as well as their serum total cholesterol level. As such, the selection of the area has no implication on the validity of our results. Furthermore, the relationship between palm oil use and serum cholesterol is a biological one and it is unlikely that it is modified by demographic and socioeconomic characteristics (neither previous studies nor ours was powered to detect such effect modification). Therefore, our results are fairly generalizable to similar peri-urban areas and possibly to rural and urban populations that use palm oil as well.

Measurements:
To make it clear I suggest to divide these paragraph in exposures, serum total cholesterol and confounders measurements.
We have revised the Methods to separate these sub-sections (Pages 7, 8).

Is the questionnaire validated?
The questionnaire was designed based on similar questionnaires in the National Health and Nutrition Examination Survey in the US as well as other standard questionnaires as detailed in the Methods. We evaluated the questionnaire in a one-week pilot study in the same area (but in another street/neighborhood). For validation of FFQ please see response to comment #3 from reviewer 1.

Since several dietary items were available, why have you only chosen certain group of food. It would be also interesting to see how other specific dietary intake of dairy or meat are related to serum cholesterol in this population.
Considering the small sample size of the study, we had to make choices and include only potentially strong confounders in our multivariable regression.

Moreover, the authors explained how the nutrients intake was calculated but I think it is more relevant for the purpose of the study to define how they treated the dietary variables used as well how question on use of oils was formulated. ¨
We have added the exact wording of the question on oil use. We have clearly explained in the Methods how the dietary variables were created and used in the regression models (Page 7).

Page 8 Line 7: I would suggest to move all this paragraph on the statistical analysis since are covariates used to adjust crude relations between diet and serum cholesterol. Moreover, I suggest to state clearly which of these factors were used as covariates
It is not clear to us why the paragraph on measurement of covariates should fall under statistical analysis. We have rephrased this section to clearly state what covariates were adjusted for (Page 8).

About the wealth index, please explain the reason to use a statistical method of difficult interpretation to create a covariate. Then, please give description how PC were retained
Measurement of socioeconomic status using indicators such as income is not appropriate in this population due to high rates of self-employment and an informal economy. Measurement of assets is a more comprehensive way of determining wealth and is often the only feasible option. We used Principal Components Analysis to create a single indicator of wealth out of 36 asset variables, which

is the only possible way to adjust for wealth in a small study like ours and is a fairly standard approach in analysis of multi-component constructs such as wealth. We retained only the first principal component to create the household wealth index. Since our analysis uses the wealth index to rank participants into tertiles of wealth, the actual interpretation of the wealth index scores does not have a bearing on our analyses.

Page 8 line 36-39: you describe your exclusion factors in the results section. Please move it there. We prefer to explain how we selected participants in the Methods and then explain what the results of the selection was in the Results as is common in many epidemiological studies.

Page 8 line 41: the author use linear regression to estimate the relation between diet and serum cholesterol. However, it really hard to understand which factors were used as exposure and which as covariates.
We have revised the Introduction to clearly explain the three main exposures and have revised the Methods to calrify which covariates were adjusted for (Pages 5, 8).

Moreover, please explain how the adjustment for interviewers was done. Please rephrase the content of this paragraph.
The only possible way to adjust for interviewer is to include it as a categorical variable in the regression. As such, we don't believe adding this to the paper is necessary.

RESULTS:

Results are not presented clearly. Sometimes tables and figures are not reflecting the aim of this study and it seems that the original aim of this project was to explore several factors related to serum cholesterol and not diet.
We respectfully disagree with the reviewer. From the start, we had hypothesized that the type of cooking oil is a major determinant of serum cholesterol and other dietary factors such as meat, fish and fruit and vegetable intake are strong determinants as well. We have revised the Introduction to make this clearer (Page 5).

Table 1: I would suggest to drop the column oil palm. Since it is a results I would suggest to put it in another table after table 2.
Table 2 is our main result table and therefore includes the results on palm oil use which was our main hypothesis.
Moreover, I suggest to give more emphasis to serum cholesterol distribution (maybe histogram of the distribution)
We do not believe a histogram will add much value over the information already provided in Table 1. For your reference, please see the histogram below:

Page 9 Line 21-41: it is not clear that all this paragraph is describing the table 1.
We are not sure what paragraph this refers to since there are no line numbers in the submitted manuscript. Nevertheless, we have checked that all paragraphs refer to appropriate Tables and Figures.

Table 2: This should be the table of results of the relation between diets and serum cholesterol. But title of the table does not report it.
We have modified the title for Table 2 to reflect this.

I suggest to have only results of the relation between diet and serum total cholesterol (crude and

adjusted). Results are presented unclearly with exposure and covariates in the same table.
We have changed the structure of the table with dietary determinants at the top and covariates in the bottom rows of the table. We prefer presenting the coefficients for the covariates in the table since it may be potentially useful information for other researchers.

Moreover, if possible I would suggest to categorize alcohol consumption (none, low, moderate and heavy).
We re-categorized alcohol consumption as suggested and ran a sensitivity analysis with the new alcohol variable. Less than 10% of the study sample falls into each of the categories of moderate and heavy consumption. The sensitivity analyses changed neither the strength nor the statistical significance of our conclusions. As in the original analysis, we did not find alcohol consumption to determine or confound the association between diet and serum total cholesterol.

Still, I would suggest to have another table with the distribution (and possibly correlation) between several covariates/variables and total serum cholesterol.
We believe all bivariate relationships with serum total cholesterol have been adequately described in column 1 of Table 2.

Page 13: The author should comment also width of the confidence interval.
We have added statistical power as one of the limitations in the Discussion which should directly address this concern about the width of the confidence intervals (Pages 19, 20).

DISCUSSION

Several part are redundant
It is not clear which parts this comment refers to. We have revised the Discussion to remove potential redundancies.

Page 16 Line 32-36: Please, explain why insufficient statistical power and measurements should be applicable only to fish intake?
We have added insufficient statistical power to our limitations and revised this sentence to clarify that the statistical power would be lower when the expected effect size is smaller or the error in exposure measurement is larger. Please see response to comment #3 from reviewer 1. (Pages 19, 20).

Page 15 Line 40-41: this sentence is redundant with the sentence in line 32-34
We are not sure what sentence this refers to since there are no line numbers in the submitted manuscript. We have nevertheless reviewed the entire page for possible redundancy.

Page 17 Line 5: I would add the small sample and how this might have affected the results.
Please see response above to the second comment on the Discussion.

Page 17 Line 45: I would make shorter your discussion on interventional program. And I would move it before the limitation and strength.
We have trimmed down our discussion on intervention (Pages 21, 22). We, however, prefer to finish the paper with the global implications of the results as is standard practice in many scientific journals.

Page 18 Line 12: it should be your conclusion but it is not clear. Please make it shorter, and try to don t leave the main focus of your study.
We have rephrased our conclusion and carved it out into a separate shorter paragraph (Pages 21, 22).

VERSION 2 – REVIEW

REVIEWER	Erkki Vartiainen
	National Institute for Health and Welfare
REVIEW RETURNED	31-Jan-2017

| GENERAL COMMENTS | All my suggestions as been taken into account in this new revision. |

REVIEWER	Rob M. van Dam
	Saw Swee Hock School of Public Health
REVIEW RETURNED	28-Jan-2017

| GENERAL COMMENTS | The authors have adequately addressed most comments. A few issues remain:
1. The authors refer to 'within-person CV%' and 'between-person CV%'. This is unconventional for CV% used to quantify laboratory variability. Do they mean 'within-batch CV%' and 'between-batch CV%'?
2. I may not have been sufficiently clear in my suggestion related to examining the association between intake of different fatty acids such as saturated fat as energy% in relation to serum cholesterol to put the results for palm oil in perspective. There is no need to include intake of these fatty acids simultaneously in the model that also includes palm oil use. This can be a separate multivariable model. |

REVIEWER	Laguzzi, Federica
	Unit of Cardiovascualr epidemiology, Instituet of Environmental Medicine, Karoliska Institutet, Sweden
REVIEW RETURNED	02-Feb-2017

| GENERAL COMMENTS | The manuscript has been revised according to the previous reviewer`s comments. No special comments to add. |

VERSION 2 – AUTHOR RESPONSE

Reviewer: 2
Dam, Rob
National University of Singapore, Saw Swee Hock School of Public Health
The authors have adequately addressed most comments. A few issues remain:

1. The authors refer to 'within-person CV%' and 'between-person CV%'. This is unconventional for CV% used to quantify laboratory variability. Do they mean 'within-batch CV%' and 'between-batch CV%'?
The manufacturer of the Cardiocheck PA device only reported one 'overall' CV by analyzing three different blood samples 10 times each. We have clarified this in our revised manuscript (Page 6).

2. I may not have been sufficiently clear in my suggestion related to examining the association between intake of different fatty acids such as saturated fat as energy% in relation to serum cholesterol to put the results for palm oil in perspective. There is no need to include intake of these fatty acids simultaneously in the model that also includes palm oil use. This can be a separate multivariable model.

As suggested, we have examined the effect of substituting saturated fat with unsaturated fats on serum cholesterol (see Table A). After keeping proportion of protein, carbohydrate and total fat intake constant, a 1% isocaloric substitution of unsaturated fat with saturated fat is associated with 1mg/dL (95% CI -3 to 5) higher serum total cholesterol (p-value 0.532). We have added this to the results section (Page 14).

Table A. Adjusted mean differences for serum total cholesterol (mg/dL) by nutrient composition; DUCS-HTN biomarker sub-study

Variable (n=347)	Fully-adjusted[b] mean difference (95% CI)	P Value[a]
% Protein intake	-8 (-14, -3)	0.002
% Carbohydrate intake	-3 (-13, -3)	0.001
% Total fat intake	-8 (-14, -2)	0.010
% Saturated fat intake	1 (-3, 5)	0.532
Total energy intake (Per 1000 kcal)	-4 (-10, 3)	0.293
Age		0.076
40-49	Reference	
50-59	4 (-8, 17)	
60 and above	16 (0, 31)	
Sex		<0.001
Male	Reference	
Female	23 (11, 34)	
Employment status		0.772
Unemployed	Reference	
Employed [c]	2 (-13, 17)	
Retired	9 (-15, 33)	
Household Wealth Index		0.405
1, Poorest	Reference	
2	-9 (-22, 4)	
3, Wealthiest	-3 (-17, 10)	
Physical activity tertile		0.283
1, least active	Reference	
2	1 (-13, 15)	
3, most active	9 (-8, 25)	
Alcohol consumption [d]		0.820
Non-drinker	Reference	
Drinker	2 (-13, 16)	

a F test for categorical variables (sex, employment status, alcohol consumption); t test for continuous variables (total energy intake, % nutrient intake); test of trend based on median value in each category for ordinal categorical variables (age category, wealth index tertile, and physical activity categories)
b Adjusted for covariates in column 1 as well as interviewer
c Self-employed, or government job, or job in private company

Reviewer: 1
Vartiainen, Erkki
National Institute for Health and Welfare

All my suggestions as been taken into account in this new revision.

Reviewer: 3
Laguzzi, Federica
Karolinska Institutet

The manuscript has been revised according to the previous reviewer`s comments. No special comments to add.

VERSION 3 – REVIEW

REVIEWER	Rob M. van Dam National University of Singapore
REVIEW RETURNED	11-Feb-2017

GENERAL COMMENTS	The authors have responded to the remaining comments. One issue remains. The authors have now included an analysis of saturated fat intake and serum cholesterol concentrations which is of interest. However, the multivariable model used seems to simultaneously include total energy intake, total fat, protein, carbohydrates, alcohol, and saturated fat. This is not a correct model as fat, protein, carbohydrates and alcohol are the only sources of energy: if energy intake, protein, carbohydrate and alcohol are 'kept constant' it is not possible to increase fat intake; in other words, for fat intake to be able to increase, intake of another macronutrient has to decrease and should be kept out of the multivariable model. If one is interested in saturated fat, one could include saturated fat, protein, carbohydrate, alcohol and energy intake simultaneously in the model. Then the beta for saturated fat would reflect an increase in saturated fat while decreasing unsaturated fat.

VERSION 3 – AUTHOR RESPONSE

Reviewer: 2

Please leave your comments for the authors below

Reviewer Name:Rob M. van Dam
Institution and Country: National University of Singapore
Please state any competing interests: None declared

The authors have responded to the remaining comments.

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unsaturated fat.

Our aim indeed was to keep all major sources of energy including total fat constant (as opposed to allowing total fat intake to increase) to estimate the effect of a substitution between saturated and unsaturated fats. Therefore, in our model we also included total fat. We agree with the reviewer that if we had alcohol as a continuous variable (e.g. grams per day), adjusting for total fat would have been unnecessary as it would be the only other source of total calories. However, to preserve statistical power, we only included a dichotomous variable for alcohol (drinker vs abstainer). Therefore, leaving total fat out of the model will render the substitution effect less clear. For example, a higher saturated fat intake may correspond to a higher total fat intake compensated by lower alcohol intake, which is not the aim of the model.

VERSION 4 – REVIEW

REVIEWER	Rob M. van Dam National University of Singapore
REVIEW RETURNED	24-Mar-2017

GENERAL COMMENTS	No further comments