PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	The effect of adiposity on differences in carotid plaque burden in studies conducted in Norway and Russia: a cross-sectional analysis of two populations at very different risk of cardiovascular mortality
AUTHORS	Imahori, Yume; Frost, Chris; Mathiesen, Ellisiv; Ryabikov, Andrey; Kudryavtsev, Alexander V.; Malyutina, Sofia; Kornev, Michael; Hughes, A; Hopstock, Laila; Leon, David

VERSION 1 - REVIEW

REVIEWER	Hayato Tada Kanazawa University, Japan
REVIEW RETURNED	08-Jan-2020

GENERAL COMMENTS	In this study, the authors investigated the factors associated with carotid plaque burden in 2 studies (Norway and Russia). They essentially found that adiposity (BMI and WHR) is not a critical factor which could explain the larger plaque burden in Russia compared with Norway via several statistical models. This study is well conducted. However, there are several major issues listed below. In the introduction section, the motivation focusing on adiposity seems weak. The readers may want to know why the authors are focusing on adiposity, since the results are essentially negative. Is there any “established” factors which could explain the difference between 2 groups? If there is, the how about in this study?

REVIEWER	Yamnia Cortes University of North Carolina Chapel Hill School of Nursing USA
REVIEW RETURNED	28-Jan-2020

GENERAL COMMENTS	It was a pleasure to review this work on the association between adiposity and carotid plaque in two distinct cohorts, the Know Your Heart study and Tromso 7. A strength of the manuscript is the use of the two well-established cohorts with a large sample size and that the authors also stratify results by sex. Additionally, the authors assess adiposity using body mass index, waist circumference, and waist-hip ratio. However, there are several limitations that need to be addressed so that the manuscript is suitable for publication:

	1. Abstract: a. Though there is limited space in the abstract, it is important that the reader understands the methodology clearly. Were the datasets merged/pooled, or were they analyze separately and the results compared? b. Which measure of abdominal adiposity was of interest? The results are presented for WHR, but this variable was not clarified up front. c. Please clearly state the statistical modeling used and whether any interactions were assessed or formal mediation analyses. 2. Introduction: a. The rationale for the comparison between Russia and Norway is clear. However, expand the background on the measurement of carotid plaque and the current evidence on associations between adiposity and carotid plaque. b. The introduction will be stronger if authors provide the specific measures of adiposity in the research aims (replacing "general/abdominal adiposity"). 3. Methods: a. It is important to state up front whether the datasets in this analysis were merged/pooled or analyzed separately. It may be helpful to format the methods so that there is enough information presented on each cohort. For example, present an overview of KYH followed by an overview of Tromso 7 and finally present the sample for the current analysis. b. Further information is needed on the measurement of adiposity and carotid plaque. Who performed the measurement and is there quality control data for the measures? Who performed the ultrasound and who read the images? How were images of read? Information on these variables in each of the studies will help the reader determine comparability of the variables across studies. Aside from the waist circumference were all other variables between the datasets comparable in terms of methods and units? c. Authors discuss confounders and mediators, but the methods do not list which variables were considered confounders versus mediators and how each of these were measured. Though authors refer to a prior paper, further details are needed in the current manuscript. d. The statistical analysis needs further clarification. The authors refer to mediation, but formal mediation analyses were not performed. How were the data modeled? In the tables, it would be helpful to list specific variables instead of "+confounders" or +mediators". 4. Results and Discussion: a. As mentioned above, please provide more details about the specific confounders and "mediators" in the tables and figure. Footnotes with details of the modeling will help make the figures stand alone. b. I understand why the CVD risk factors are considered potential mediators, but it may be more helpful to examine them one by one instead of including them in the model all at once. Then you may provide more discussion on changes in the parameter estimates. The discussion would then refer to them as potential mediators. It is currently written as if these are definitive mediators and that a mediation analysis was performed. Thank you again for this important work.

VERSION 1 – AUTHOR RESPONSE

Response to reviewer 1

In this study, the authors investigated the factors associated with carotid plaque burden in 2 studies (Norway and Russia). They essentially found that adiposity (BMI and WHR) is not a critical factor which could explain the larger plaque burden in Russia compared with Norway via several statistical models. This study is well conducted. However, there are several major issues listed below

We appreciate the reviewer's positive assessment of our approach.

In the introduction section, the motivation focusing on adiposity seems weak. The readers may want to know why the authors are focusing on adiposity, since the results are essentially negative.

Obesity and more adipose body composition are associated with increased risk of carotid plaque. Carotid plaque is associated with increased risk of stroke and IHD events, and could thus provide a partial explanation for why Russia compared to Western European countries such as Norway have such high CVD mortality. This is of particular interest as other common risk factors do not appear to explain much of the CVD difference between Russia and other countries.

To elaborate this, we have added a new sentence "It appears that the differences in conventional CVD risk factors such as smoking, blood pressure, and cholesterol levels do not explain this difference (1, 2) " to the first paragraph in the introduction section (page 4 line 8-9). Furthermore, we have added another sentence "To the best of our knowledge, the contribution of general or abdominal obesity to the gap in CVD burden between Russia and Western European countries has not been investigated in spite of increasing importance of obesity as a CVD risk factor." (page 4 line 15-18) to make our motivation clearer.

Is there any "established" factors which could explain the difference between 2 groups? If there is, the how about in this study?

As mentioned above, it appears that the reason behind the high CVD mortality rate in Russia compared to Western European countries has been unclear. Very heavy alcohol consumption can be a potential determinant of high CVD mortality rate in Russia. As we mentioned in the discussion section, it would have been interesting to explore this. However, measures of alcohol consumption in the two study populations were not directly comparable. We added this explanation to the discussion section (page 10 line 26-27).

Response to reviewer 2

It was a pleasure to review this work on the association between adiposity and carotid plaque in two distinct cohorts, the Know Your Heart study and Tromso 7. A strength of the manuscript is the use of the two well-established cohorts with a large sample size and that the authors also stratify results by

sex. Additionally, the authors assess adiposity using body mass index, waist circumference, and waist-hip ratio. However, there are several limitations that need to be addressed so that the manuscript is suitable for publication:

We thank the reviewer for the positive assessment of our study design and exposures. We appreciate many valuable suggestions.

Abstract 1: Though there is limited space in the abstract, it is important that the reader understands the methodology clearly. Were the datasets merged/pooled, or were they analyze separately and the results compared?

Thank you for raising this important point. We agree whether we analysed pooled datasets or separate ones are a bit confusing. We analysed the datasets both separately and pooled, and we mainly presented the result using the pooled datasets because there was not enough evidence of the interaction between the study and outcomes. To clarify this, we have revised the part of Results section. Now it reads:

“A positive association between carotid plaque burden and adiposity was found (OR of having at least one plaque per SD in WHR 1.18 (95% CI 1.06, 1.31) for men; 1.15 (1.06, 1.25) for women)) adjusted for age, smoking and education in a pooled analysis of the two studies. There was little evidence of the interaction between study and adiposity.”(page 2 line 16-17)

Abstract 2: Which measure of abdominal adiposity was of interest? The results are presented for WHR, but this variable was not clarified up front.

Thank you for pointing this out. We have now added “assessed using body mass index (BMI) and waist-to-hip ratio(WHR)” to the first paragraph of the abstract (page 2 line 4-5) so that our adiposity variables are clear up front.

Abstract 3: Please clearly state the statistical modeling used and whether any interactions were assessed or formal mediation analyses.

We agree with this and have added the statistical method to Design section (page 2 line 7). We have also shown the result of the interaction test (page 2 line 17).

Introduction 1: The rationale for the comparison between Russia and Norway is clear. However, expand the background on the measurement of carotid plaque and the current evidence on associations between adiposity and carotid plaque.

We have introduced our previous research which investigated the association between adiposity and carotid plaques and made it clearer why we chose WHR as a measure of abdominal adiposity in the present study (page 4 line 22-24).

Introduction 2: The introduction will be stronger if authors provide the specific measures of adiposity in the research aims (replacing "general/abdominal adiposity").

We appreciate the reviewer's suggestion. We have replaced general/abdominal adiposity with BMI or WHR in the last paragraph of the introduction section (page 4, line 27-28, 29, 30).

Methods 1: It is important to state up front whether the datasets in this analysis were merged/pooled or analyzed separately. It may be helpful to format the methods so that there is enough information presented on each cohort. For example, present an overview of KYH followed by an overview of Tromso 7 and finally present the sample for the current analysis.

Thank you for your valuable suggestion. We agree that the description of our methods should be modified. Now we have added a paragraph at the beginning and made it clearer that two studies were designed to be comparable in terms of cardiovascular phenotype, with the researchers from Tromsø 7 and KYH working collaboratively together at the design stage on the measurement protocols used (page 5 line 1-4). This was followed by an overview of each study and the inclusion criteria of the present study.

In some of the analyses, we use the pooled dataset while in others we did not, as it depended upon the specific question we were attempting to answer. Regarding tables 1 and 2, we analysed the two studies separately because we were interested in participant characteristics and the prevalence of carotid plaques in each study. As for tables 3 and 4, we analysed both separate dataset and pooled dataset. In the main manuscript, we mainly presented the pooled result because our interaction test between adiposity and study showed little evidence that the association between adiposity and carotid plaques was significantly different between the two countries.

We have made it more explicit by adding one sentence "We also analysed the two studies separately, but presented the result from the pooled data based on the test of interaction described below" to the result section (page 8 line 3-4).

Methods 2: Further information is needed on the measurement of adiposity and carotid plaque. Who performed the measurement and is there quality control data for the measures? Who performed the ultrasound and who read the images? How were images of read? Information on these variables in each of the studies will help the reader determine comparability of the variables across studies. Aside from the waist circumference were all other variables between the datasets comparable in terms of methods and units?

Thank you for this suggestion. Regarding the ultrasound examination, we have made it clearer by adding more detailed procedure in supplementary material and three references in the main manuscript (page 6 line 9). Because our manuscript already exceeds 3000 words, we have mainly added the detail to supplementary material to improve the readability. We believe this information helps readers to assess the comparability of the burden of carotid plaques between the two studies. Besides, we have articulated that KYH was designed to be comparable with the Tromsø 7 along with the reference (page 5 line 1-4).

Regarding the measurement of adiposity, recent studies have shown high inter-observer reliability of waist circumference and WHR measurements (3, 4). Furthermore, it has been shown that training improves the reliability of these measurements (5). As mentioned in our methods section (page 5 line 29), anthropometric measures in the two studies were conducted by trained staff following the protocol. For these reasons, we believe the assessment of anthropometric measures in the two studies is reasonably reliable.

Methods 3: Authors discuss confounders and mediators, but the methods do not list which variables were considered confounders versus mediators and how each of these were measured. Though authors refer to a prior paper, further details are needed in the current manuscript.

We agree that we should provide more detailed information on measurement of metabolic CVD risk factors. We have added the detail to supplementary material. Potential confounders and mediators are listed in our methods section (page 6 line 27-30). How information on age, smoking, educational attainment, medical history was obtained is described in our method section (page 6 line 3-7).

Methods 4: The statistical analysis needs further clarification. The authors refer to mediation, but formal mediation analyses were not performed. How were the data modeled? In the tables, it would be helpful to list specific variables instead of "+confounders" or +mediators

Our statistical approach is outlined in detail on pages 6-7. Our description of how we looked at potential mediators is described on bottom of page 6 – line 5 page 7. We did not undertake formal mediation analysis. In the Tables, the footnotes describe very explicitly what variables were used in each regression model. We hope this is sufficiently explicit.

Our statistical approach is outlined in detail from on pages 6-7. Our description of how we looked at

Results/Discussion 1 : As mentioned above, please provide more details about the specific confounders and "mediators" in the tables and figure. Footnotes with details of the modeling will help make the figures stand alone.

For figure, we have added footnotes to explain confounders and mediators. For tables 3 and 4, information is already included in the footnotes.

Result. I understand why the CVD risk factors are considered potential mediators, but it may be more helpful to examine them one by one instead of including them in the model all at once. Then you may provide more discussion on changes in the parameter estimates. The discussion would then refer to them as potential mediators. It is currently written as if these are definitive mediators and that a mediation analysis was performed.

Thank you for this suggestion. We originally considered this, but concluded that it was unlikely that we could robustly quantify the strength of each of the separate potential mediation pathways. Once one starts to attempt this issues to do with differences in measurement error of the covariates becomes important, and the different potential confounding between the potential mediators needs to be accounted for. In this paper, we believe that it is sufficient to note that jointly the potential mediators have an important effect.

1. Kuulasmaa K, Tunstall-Pedoe H, Dobson A, Fortmann S, Sans S, Tolonen H, et al. Estimation of contribution of changes in classic risk factors to trends in coronary-event rates across the WHO MONICA Project populations. Lancet (London, England). 2000;355(9205):675-87.

2. Averina M, Nilssen O, Brenn T, Brox J, Kalinin AG, Arkhipovsky VL. High cardiovascular mortality in Russia cannot be explained by the classical risk factors. The Arkhangelsk Study 2000. European journal of epidemiology. 2003;18(9):871-8.

3. Mason S, Knowles E, Colwell B, Dixon S, Wardrope J, Gorringe R, et al. Effectiveness of paramedic practitioners in attending 999 calls from elderly people in the community: cluster randomised controlled trial. BMJ (Clinical research ed). 2007;335(7626):919.

4. Chen MM, Lear SA, Gao M, Frohlich JJ, Birmingham CL. Intraobserver and interobserver reliability of waist circumference and the waist-to-hip ratio. Obesity research. 2001;9(10):651.

5. Albrektsen G, Heuch I, Lochen ML, Thelle DS, Wilsgaard T, Njolstad I, et al. Lifelong Gender Gap in Risk of Incident Myocardial Infarction: The Tromso Study. JAMA internal medicine. 2016.

VERSION 2 – REVIEW

REVIEWER	Hayato Tada Kanazawa University, Japan
REVIEW RETURNED	17-Mar-2020

GENERAL COMMENTS	The reviewer completed the checklist but made no further comments.

REVIEWER	Yamnia I. Cortes University of North Carolina at Chapel Hill
REVIEW RETURNED	30-Mar-2020

GENERAL COMMENTS	It was a pleasure to review this revised manuscript, which is using two large epidemiological studies in Russia and Norway to compare associations between general adiposity and carotid plaque. In this revision the authors have more clearly defined the research aims and methods. The introduction and methods provide important definitions of the general adiposity measures and carotid plaque. The supplemental material also provides important information on how the authors dealt with each study using different ultrasound equipment to assess carotid plaque. Moreover, there is a clear overview of each of the datasets and how these were analyzed to answer the research questions. The revision provides a more detailed description of the statistical models with a distinction between those variables that are considered confounders versus potential mediators. The rationale for the data analysis plan is also sound. My prior comments/concerns have been addressed.