

PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (<http://bmjopen.bmj.com/site/about/resources/checklist.pdf>) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Associations of movement behaviours and dietary intake with arterial stiffness: results from the ORISCAV-LUX 2 cross-sectional study
AUTHORS	Backes, Anne; Collings, Paul J; Portugal, Berta; Quintero, Lilly Carina; Vahid, Farhad; Le Coroller, Gwenaëlle; Malisoux, Laurent; Group authorship, ORISCAV-LUX study group

VERSION 1 – REVIEW

REVIEWER	McEwen, Bradley Southern Cross University
REVIEW RETURNED	10-Mar-2024

GENERAL COMMENTS	<p>Dear Dr Backes and colleagues,</p> <p>The manuscript you submitted to BMJ Open titled “Associations of movement behaviours and dietary intake with vascular health: Results from the ORISCAV-LUX 2 cross-sectional study” is novel and interesting. The manuscript has sufficient impact and adds to the knowledge base. The manuscript provides potential insights of the associations between movement behaviours and dietary intake with pulse wave velocity, as well as sex differences of nutrient profiles. Arterial stiffness has a significant impact on cardiometabolic syndrome and the progression of cardiovascular disease. Nutrition and physical activity play significant roles in the improvement of health. This cross-sectional study investigated the effect of both the intake of dietary antioxidants and trace elements and a physically active lifestyle on arterial stiffness. The findings of this study highlight the significance of considering both movement behaviours and dietary antioxidant intake in cardiovascular health assessments. Of interest is the differences of selenium and vitamin C reducing PWV in women and polyphenols and selenium reducing PWV in men. The commonality between the effects on PWV on women and men being selenium. The noted limitation of the declared nutrient intakes exceeding the common recommendations is useful as future research could look at the differences between people with nutrient deficiencies and nutrient proficiencies.</p> <p>This trial is very exciting and novel.</p>
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REVIEWER	Lin, Pao-Hwa Duke University Medical Center, Department of Medicine
REVIEW RETURNED	14-Mar-2024

GENERAL COMMENTS	<p>This manuscript reports the results of a cross-sectional analysis of the relationship between physical activity, sedentary behavior, dietary antioxidants and trace elements intake with vascular health as indicated by arterial stiffness and if the relationship varied by sex. The manuscript is very well prepared. There are only a couple of minor comments for their considerations.</p> <ol style="list-style-type: none"> 1. Lines 119-119, if the participants wear the actigraphTM for 7 consecutive days and the first day and the last half-day were excluded, how could data of six complete days be obtained? The math doesn't seem to add up. 2. It would be more helpful to give even more specific examples of the type of activities that would be considered "longer duration at higher activity intensities as well as a more pronounced long-range temporal correlation in activity fluctuations..."
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REVIEWER	Ullrich, Antje University of Greifswald Institute of Community Medicine, Department of Social Medicine and Prevention
REVIEW RETURNED	15-Mar-2024

GENERAL COMMENTS	<p>Thank you for giving me the opportunity to review this manuscript. This cross-sectional study investigated the association between accelerometer-based movement behaviour, dietary intake and arterial stiffness among individuals from the general population in Luxembourg. In general, this manuscript has strengths and novel insights that make it of relevance in terms of advancing the field of understanding the association of different parameters of movement behaviours, dietary intake, and arterial stiffness. Although the study focuses on an important topic, uses advanced methodological and statistical approaches, has an apparently large sample size and is overall well written, it raises important questions, some of which are of major importance. Therefore, I cannot recommend the manuscript for publication without reviewing it again after revision. I have outlined my major and minor concerns below.</p> <p>Major compulsory revisions</p> <p>Background <input type="checkbox"/> Line 61-63: Is there detailed information in the literature on the (short- or long-term) associations between different PA intensities or measures of SB with arterial stiffness (PWV), arteroclerosis or CVDs? If so, this should be highlighted in the background section to relate the results of the different WIPAB to the outcome of this study in the discussion section.</p> <p><input type="checkbox"/> Please include a statement if there are information in the literature on sexspecific differences on dietary patterns and cardiovascular health.</p> <p><input type="checkbox"/> Arterial stiffness is related to age and other lifestyle behaviours (e.g., smoking, alcohol consumption). This should be mentioned in the background section and discussed in the discussion section as well. In</p>
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	<p>addition, the authors should explain why there are focused on sexstratified analysis and not included other related factors as an interactional term in the model.</p> <p>Methods <input type="checkbox"/> Please provide information on the steps taken to select the study participants. This includes a systematic analysis of the individuals who participated or did not participate in certain examinations (selection steps, e.g., recruitment, accelerometry, measurement of PWV). This allows a possible bias in the selection of study participants to be estimated to a certain extent (e.g., self-selection bias, non-responder bias). In this way, extrapolated generalization from a sample to a population can be avoided. This aspect should be highlighted in more detail in the discussion section.</p> <p><input type="checkbox"/> To check the robustness of the study results, I would suggest that the authors perform an analysis using random forest (regression). These results could be included as a supplementary file if necessary and strengthens the informative value of the study result.</p> <p><input type="checkbox"/> Line 169-170: "Elastic-net analysis is expected to yield superior results...". Have the authors checked the results for ridge, lasso and elastic-net regression separately to compare the results with each other?</p> <p>BMJ Open Minor essential revisions</p> <p>Title <input type="checkbox"/> I recommend to revise the title of the manuscript to be more precise. Please use the term "arterial stiffness" instead of "vascular health".</p> <p>Background <input type="checkbox"/> Line 65: Please indicate that the daily contribution of MVPA (6%) was determined in a study with the same population. There are other population-based studies that report lower absolute and relative daily contributions of MVPA. Selection (sample) bias should be considered when reporting these results.</p> <p><input type="checkbox"/> Line 65-69: Please include an introduction why analysing associations between detailed information on movement behaviour (e.g., WIPAB) and cardiovascular health is important. Is there information in the literature which of these parameters might be more beneficial or detrimental for arterial stiffness than others?</p> <p>Methods <input type="checkbox"/> Please include some information on the recruitment strategies because this could influence the response rate and the selection of study participants.</p> <p><input type="checkbox"/> Line 101: Please provide the unit of measurement (PWV).</p> <p><input type="checkbox"/> Line 127-129: Please explain why you used SB bouts with a duration of</p>
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	<p>over 30min and no other parameters such as <10min, over 10 to 30min?</p> <ul style="list-style-type: none"> <input type="checkbox"/> Please include data proceeding information such as what is the definition of 1 SB bout. <input type="checkbox"/> Please explain why accelerometry wear time was not included as a covariate in the analysis model. <input type="checkbox"/> Line 128: The authors used MVPA bout length. Which cut-off value was used to determine MVPA? <input type="checkbox"/> Line 141: Please report psychometric characteristics of the questionnaire FFQ. <input type="checkbox"/> Line 155-156: Please include how medication intake and family history of disease were categorized. <input type="checkbox"/> Line 167: What does “For each patient groups” means; that seems not correct. Please revise the sentence. <input type="checkbox"/> Which size was the training- and the test-sample? Please include this information. <p>Discussion <input type="checkbox"/> Line 223-224: Because of the several selection steps in this sample, I would avoid using the term “in a general adult population”.</p> <ul style="list-style-type: none"> <input type="checkbox"/> Line 234: Please introduce the abbreviations; “SOD and GPx”. General aspects <input type="checkbox"/> Please use consistent terminology (e.g., movement behaviour, physical behaviour; arterial, aortic stiffness). <input type="checkbox"/> Is there an original protocol for the study that can be included as a supplementary file? <input type="checkbox"/> Please avoid using words such as “effect” (line 77) or “improved arterial health” (line 240), because of the cross-sectional study design.
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VERSION 1 – AUTHOR RESPONSE

REVIEWER 1: COMMENTS TO THE AUTHOR

Dear Dr Backes and colleagues,

The manuscript you submitted to BMJ Open titled “Associations of movement behaviours and dietary intake with vascular health: Results from the ORISCAV-LUX 2 cross-sectional study” is novel and interesting. The manuscript has sufficient impact and adds to the knowledge base. The manuscript provides potential insights of the associations between movement behaviours and dietary intake with pulse wave velocity, as well as sex differences of nutrient profiles. Arterial stiffness has a significant impact on cardiometabolic syndrome and the progression of cardiovascular disease. Nutrition and physical activity play significant roles in the improvement of health. This cross-sectional study investigated the effect of both the intake of dietary antioxidants and trace elements and a physically active lifestyle on arterial stiffness. The findings of this study highlight the significance of considering both movement behaviours and dietary antioxidant intake in cardiovascular health assessments. Of interest is the differences of selenium and vitamin C reducing PWV in women and polyphenols and selenium reducing PWV in men. The commonality between the effects on PWV on women and men being selenium. The noted limitation of the declared nutrient intakes exceeding the common

recommendations is useful as future research could look at the differences between people with nutrient deficiencies and nutrient proficiencies.

This trial is very exciting and novel.

Thank you very much for sparing your time to review our manuscript and for your kind comments.

REVIEWER 2: COMMENTS TO THE AUTHOR

This manuscript reports the results of a cross-sectional analysis of the relationship between physical activity, sedentary behaviour, dietary antioxidants and trace elements intake with vascular health as indicated by arterial stiffness and if the relationship varied by sex. The manuscript is very well prepared. There are only a couple of minor comments for their considerations.

Thank you for making the effort to review our manuscript and for your helpful feedback.

1. Lines 119-119, if the participants wear the actigraphTM for 7 consecutive days and the first day and the last half-day were excluded, how could data of six complete days be obtained? The math doesn't seem to add up.

Thank you for your comment. As the starting and end time of the recordings differed among the participants, the first half-day (before midnight) and the last half-day (after midnight) from the seven consecutive days were excluded in order to obtain up to six complete days. We adapted the corresponding sentence to be more specific:

“Acceleration data between the first and last midnight was used” (line 130-133).

2. It would be more helpful to give even more specific examples of the type of activities that would be considered “longer duration at higher activity intensities as well as a more pronounced long-range temporal correlation in activity fluctuations...”.

Thank you for pointing this out. We adapted the corresponding paragraph to give more specific examples:

“This implies that a longer duration at higher activity intensities (e.g. brisk walking, jogging, lawn mowing or washing windows) as well as a more pronounced long-range temporal correlation in activity fluctuations (i.e., regular exercise routine with repetitive patterns over time) may have a beneficial impact on arterial health in women” (line 287-290).

REVIEWER 3: COMMENTS TO THE AUTHOR

Thank you for giving me the opportunity to review this manuscript. This cross-sectional study investigated the association between accelerometer-based movement behaviour, dietary intake and arterial stiffness among individuals from the general population in Luxembourg.

In general, this manuscript has strengths and novel insights that make it of relevance in terms of advancing the field of understanding the association of different parameters of movement behaviours, dietary intake, and arterial stiffness. Although the study focuses on an important topic, uses advanced methodological and statistical approaches, has an apparently large sample size and is overall well written, it raises important questions, some of which are of major importance. Therefore, I cannot recommend the manuscript for publication without reviewing it again after revision. I have outlined my major and minor concerns below.

Thank you for your detailed review and valuable comments. We hope that we have properly addressed all your questions and comments.

Major compulsory revisions

Background

- Line 61-63: Is there detailed information in the literature on the (short- or long-term) associations between different PA intensities or measures of SB with arterial stiffness (PWV), atherosclerosis or CVDs? If so, this should be highlighted in the background section to relate the results of the different WIPAB to the outcome of this study in the discussion section.

Previous research mainly focused solely on light, moderate-to-vigorous PA or exercise and its association with cardiovascular health [1-3]. For instance, it has been found that aerobic exercise lasting more than 8 weeks, but not exercise lasting 4-8 weeks, significantly improved PWV in healthy older adults, though the certainty of evidence was very low, and effects were not observed in older adults with existing diseases [2]. Besides, literature suggests a dose-dependent relationship between exercise intensity and arterial stiffness improvement, with higher intensities enhancing the benefits of aerobic exercise on arterial stiffness [4]. However, to the best of our knowledge, no research has investigated all-encompassing variables that capture the entire spectrum of movement behaviours and their relationship to cardiovascular health. Therefore, the present study aims to investigate how a physically active lifestyle, and more specifically which of the components, are related to arterial stiffness, while mutually adjusting for dietary patterns.

We added this information to the background section:

“Regular PA and a reduced sedentary behaviour (SB) are associated with decreased cardiovascular mortality [3] and with a lower risk of atherosclerosis and CVDs such as myocardial infarction and stroke [5, 6]. The literature suggests a dose-dependent relationship between exercise intensity and arterial stiffness improvement, with higher intensities enhancing the benefits of aerobic exercise on arterial stiffness [4]. However, to the best of our knowledge, no research has investigated movement behaviour indicators that encompass the full spectrum of intensities and behaviours during waking hours and their relationship to cardiovascular health. These indicators may provide more informative insights than focusing solely on moderate-to-vigorous PA (MVPA), which has been found to constitute only 6% of daily time in a study with the same population [7] “ (line 63-71).

- Please include a statement if there are information in the literature on sex-specific differences on dietary patterns and cardiovascular health.

Thank you for the comment. Indeed, a reference on sex-related differences on dietary patterns and cardiovascular health should be added to the corresponding paragraph:

“Previous research has already provided evidence of sex-related differences in markers of arterial stiffness [8-10], movement behaviours [11], dietary patterns [12] and their associations with cardiovascular risk factors [13, 14]” (line 76-78).

- Arterial stiffness is related to age and other lifestyle behaviours (e.g., smoking, alcohol consumption). This should be mentioned in the background section and discussed in the discussion section as well. In addition, the authors should explain why there are focused on sex-stratified analysis and not included other related factors as an interactional term in the model.

We used sex-stratified analysis because the literature supports differential clinical characteristics in the development of arterial stiffness between men and women, indicating the involvement of sex-specific mechanisms [8-10]. We hypothesised that the relationship between PA or dietary pattern and PWV may differ between sexes. Including an interaction term would have complicated the models, making interpretation more difficult. Therefore, we considered sex as an effect modifier rather than including an interaction term in the analysis. Additionally, multiple potential confounders, including lifestyle factors such as smoking, were adjusted for in the analysis.

Methods

- Please provide information on the steps taken to select the study participants. This includes a systematic analysis of the individuals who participated or did not participate in certain examinations (selection steps, e.g., recruitment, accelerometry, measurement of PWV). This allows a possible bias in the selection of study participants to be estimated to a certain extent (e.g., self-selection bias, non-responder bias). In this way, extrapolated generalization from a sample to a population can be avoided. This aspect should be highlighted in more detail in the discussion section.

Detailed information on the participants included in this study has been provided in the method section. Only those with missing, erroneous or implausible data were excluded from the main database. The main paper of the ORISCAV-LUX 2 study, cited in the study population paragraph,

analysed population coverage and sample representativeness, concluding that the ORISCAV-LUX datasets represent a relevant tool for epidemiological research in Luxembourg [15]. However, it is important to note that the ORISCAV-LUX 2 sample differs in some respects from the general Luxembourg population. As highlighted in the limitation paragraph, the ORISCAV-LUX study sample was generally healthier than the general population, a phenomenon known as “healthy participant bias”. This bias is common and also applies to large population-based studies, including the UK Biobank, where participants reported fewer health conditions and were less likely to be current smokers compared to the general population [16]. Despite these differences, the present study had a high sample size relative to the total population of Luxembourg, with variability in exposures and outcomes. These features are crucial for producing valid and generalizable information about associations between exposure and disease [17].

In order to better visualise the different exclusion steps, a flowchart has been added to the supplementary material.

“A flowchart of the participants’ inclusion process can be found in the supplementary material (Figure S1)” (line 106-107).

- To check the robustness of the study results, I would suggest that the authors perform an analysis using random forest (regression). These results could be included as a supplementary file if necessary and strengthens the informative value of the study result.

Random forest and elastic-net models are both popular machine learning models, but are fundamentally different in their approach, underlying assumptions and use cases. Consequently, the results of these models cannot be directly compared. Elastic-net models should be preferred if feature selection and model interpretability are the main goal. In contrast, random forest models excel in prediction and classification tasks. Additionally, elastic-net models are expected to better handle multicollinearity compared to random forest models as they incorporate both L1 and L2 regularisation (combining the penalties of Lasso and Ridge regression), which helps in handling multicollinearity by shrinking coefficients and selecting relevant features.

- Line 169-170: “Elastic-net analysis is expected to yield superior results...”. Have the authors checked the results for ridge, lasso and elastic-net regression separately to compare the results with each other?

Indeed, we have compared mean squared errors of LASSO, elastic-net and ridge regression models in a preliminary analysis. The Elastic-net model presented thereby the lowest error. As other studies also confirmed the expectation of superior results of the elastic-net models, compared to LASSO and ridge regression, especially when analysing highly correlated predictors [18, 19], we did not include the results of the preliminary analysis in the present paper.

Minor essential revisions

Title

- I recommend to revise the title of the manuscript to be more precise. Please use the term "arterial stiffness" instead of "vascular health".

Thank you for the recommendation. Indeed, we do agree and changed the title to:

“Associations of movement behaviours and dietary intake with arterial stiffness: Results from the ORISCAV-LUX 2 cross-sectional study”

Background

- Line 65: Please indicate that the daily contribution of MVPA (6%) was determined in a study with the same population. There are other population-based studies that report lower absolute and relative daily contributions of MVPA. Selection (sample) bias should be considered when reporting these results.

Thank you for your comment. We added the information that these 6% were found in a study with the same population:

“These indicators may provide more informative insights than focusing solely on moderate-to-vigorous PA (MVPA), which has been found to constitute only 6% of daily time in a study with the same population” (line 67-71).

- Line 65-69: Please include an introduction why analysing associations between detailed information on movement behaviour (e.g., WIPAB) and cardiovascular health is important. Is there information in the literature which of these parameters might be more beneficial or detrimental for arterial stiffness than others?

As mentioned in our reply to your preceding comment, current research often focuses solely on MVPA and its association with cardiovascular health. However, this approach overlooks many dimensions of the movement behaviour. A more holistic approach may be more appropriate. Therefore, the present study aims to address this issue by investigating which movement behaviour variables are relevant for arterial health. This argumentation can be found in the paragraph from line 61 to 73.

Methods

- Please include some information on the recruitment strategies because this could influence the response rate and the selection of study participants.

Detailed information on the ORISCAV-LUX 2 study sample can be found in the cited paper from Alkerwi et al. [15]. Population coverage and sample representativeness have been analysed and it has been concluded that the ORISCAV-LUX datasets represents a valid tool for epidemiological studies in Luxembourg [15]. Please, see also our reply to your comment on pages 5-6 of this rebuttal letter.

We added this information to the corresponding paragraph:

“Population coverage and sample representativeness have been analysed and it has been concluded that the ORISCAV-LUX dataset represents a valid tool for epidemiological studies in Luxembourg” (line 95-97).

Additionally, a flowchart of the participants included in the present study has been added to the supplementary material (Figure S1).

We have already acknowledged in the limitations section that ORISCAV-LUX 2 study participants were generally healthier than non-participants, and we referenced two supporting studies. (line 339-343).

- Line 101: Please provide the unit of measurement (PWV).

Thank you for pointing this out. The unit of measurement has been added to the corresponding sentence:

“The validated Complior® instrument (ALAM medical, Vincennes, France) was used to assess PWV (m/s)” (line 109-110).

- Line 127-129: Please explain why you used SB bouts with a duration of over 30min and no other parameters such as <10min, over 10 to 30min?

The selected threshold of 30 minutes is somewhat arbitrary, but has been used in a previous study that we conducted on substituting device-measured sedentary time with alternative 24-hour movement behaviours (as well as in other studies on cardiovascular health). The corresponding reference has been added to clarify this:

“Additionally, we computed the number of prolonged sedentary episodes of a duration higher than 30 minutes (SB bouts >30 min) [20], the median duration of sedentary episodes (median SB bout length) and the median MVPA bout length, which describe the activity accumulation at specific intensities” (line 140-143).

- Please include data proceeding information such as what is the definition of 1 SB bout.

A “bout” is a common term to describe activity or inactivity episodes of a certain duration. Hence, in our case, one SB bout does not have a specific defined length but differs based on the activity

analysed. The main publication providing detailed information on all movement behaviour variables used has been cited at the end of that paragraph (line 151-152). The corresponding paragraph has been adjusted to clarify this (please see comment above).

- Please explain why accelerometry wear time was not included as a covariate in the analysis model.

The study participants demonstrated excellent compliance with the habitual movement assessment, and the near-continuous (24-hour) wear protocol minimised missing data. Median (IQR) wear time was 1440 (1435 to 1440) min/d [11]. Therefore, we decided to not include accelerometer wear time as covariate in the analysis model.

- Line 128: The authors used MVPA bout length. Which cut-off value was used to determine MVPA?

Thanks for pointing this out. We added the thresholds used to estimate the time spent in sedentary and MVPA:

“Validated thresholds were used to estimate the average daily awake time spent in sedentary (<44.8 mg) and MVPA (>100.6 mg) [21]” (line 132-133).

- Line 141: Please report psychometric characteristics of the questionnaire FFQ.

The reference cited confirms that the Food Frequency Questionnaire is a validated tool to assess dietary intake. We adapted the corresponding sentence to clarify this:

“Dietary intake was determined based on the quantity and frequency of food consumption reported in the validated 174-item food frequency questionnaire (FFQ) [22]” (line 155-156).

- Line 155-156: Please include how medication intake and family history of disease were categorized.

The specific categorisations were added to the corresponding sentence:

“In addition, the use of any medication for hypertension, diabetes or dyslipidemia (yes/no) as well as their family history of CVD or diabetes (yes/no/unknown) were assessed by a questionnaire” (line 171-173).

- Line 167: What does “For each patient groups” means; that seems not correct. Please revise the sentence.

Indeed, the current wording might be confusing. We revised the sentence:

“For women and men, separate generalized linear regression models with embedded elastic-net variable selection approach were built to explore how movement behaviour and dietary patterns related to PWV” (line 184-186).

- Which size was the training- and the test-sample? Please include this information.

Training and test samples are primarily necessary when the main objective involves the prediction of new data or the comparison of different models. A preliminary comparison of the mean squared errors of LASSO, elastic-net and ridge regression models was conducted using a 70 to 30 percent train-test split. As, in this study, the main objective of the elastic-net models was to select the most robust variables, no training- and test-sample has been used in the main analysis. However, the selection of the most robust variables involved 1000 iterations, each incorporating internal 10-fold cross-validation to tune the hyperparameters alpha and lambda.

Discussion

- Line 223-224: Because of the several selection steps in this sample, I would avoid using the term “in a general adult population”.

We changed the sentence to:

“The primary aim of this study was to investigate the associations of movement behaviours and dietary intake with vascular health, measured using an indicator of arterial stiffness (PWV), in an adult population of Luxembourg” (line 240-241).

- Line 234: Please introduce the abbreviations; “SOD and GPx”.

The definitions of the abbreviations have been added:

“The findings of the review indicated that regular PA has a positive impact on glutathione levels in body fluids and promotes the activities of antioxidant enzymes such as superoxidase dismutase (SOD) and glutathione peroxidase (GPx), which are dependent on essential minerals like zinc and selenium, respectively” (line 249-252).

General aspects

- Please use consistent terminology (e.g., movement behaviour, physical behaviour; arterial, aortic stiffness).

Thanks for pointing this out. We adapted the corresponding sentences:

“Limitations include the absence of a posture allocation algorithm to mitigate the misclassification of movement behaviours, such as standing as sedentary time, which is common with wrist-worn accelerometers” (line 38-40).

“Another limitation of this study is the absence of a posture allocation algorithm to mitigate the misclassification of movement behaviours, such as standing as sedentary time, which is common with wrist-worn accelerometers” (line 343-346).

“Nevertheless, previous work on long-term effects of PA and SB on changes in arterial stiffness, have demonstrated that spending more time engaged in PA and minimising SB are associated with a slower age-related progression of arterial stiffness” (line 299-301).

- Is there an original protocol for the study that can be included as a supplementary file? The protocol of the ORISCAV-LUX2 study was not published elsewhere. However, a previous manuscript aimed to “summarise the different sampling strategies adopted in the ORISCAV-LUX2 study, with a focus on the evaluation of population coverage and the sample representativeness” [15].

- Please avoid using words such as “effect” (line 77) or “improved arterial health” (line 240), because of the cross-sectional study design.

Indeed, thanks for pointing this out. We replaced these terms with more appropriate wording:

“The findings reveal diverse associations between PA, SB, dietary intake, and PWV, with distinct patterns observed in men and women” (line 15-16).

“Therefore, the present study aimed to investigate how intake of dietary antioxidants and trace elements (i.e. beta-carotene, vitamins A, C, E, polyphenols, selenium and zinc) and a physically active lifestyle (assessed using WIPAB) are related to arterial stiffness, with mutual adjustments for each other” (line 83-86).

“In the present study, the relationship between the intake of antioxidant-rich nutrients and arterial stiffness, independent of an active lifestyle, could also be demonstrated. In particular, the important role of selenium in combination with polyphenols and vitamin C could be outlined, with distinct patterns observed in men and women” (line 263-257).

References

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VERSION 2 – REVIEW

REVIEWER	Ullrich, Antje University of Greifswald Institute of Community Medicine, Department of Social Medicine and Prevention
REVIEW RETURNED	27-Jun-2024

GENERAL COMMENTS	<p>I thank the authors for addressing my concerns in detail. The revisions to the manuscript have largely addressed my major concerns and improved the manuscript; only a few minor issues remain, which are addressed below:</p> <p>1. Line 169-170: “Elastic-net analysis is expected to yield superior results...”. Have the authors checked the results for ridge, lasso and elastic-net regression separately to compare the results with each other? Indeed, we have compared mean squared errors of LASSO, elastic-net and ridge regression models in a preliminary analysis. The Elastic-net model presented thereby the lowest error. As other studies also confirmed the expectation of superior results of the elastic-net models, compared to LASSO and ridge regression, especially when analysing highly correlated predictors [18, 19], we did not include the results of the preliminary analysis in the present paper.</p> <p>As you have checked the results in a preliminary analysis, I would recommend adapting the wording of the sentence and avoiding the words "is expected to yield".</p> <p>2. The authors emphasize the influence of sex hormones on arterial stiffness. Therefore, it might be worth adding a sentence in the discussion section focusing on arterial stiffness and menopause in women, considering a recently published paper by O`Neill et al. (2024). https://doi.org/10.1016/j.maturitas.2023.107900</p> <p>3. Line 35: Please use 'cardiovascular diseases' instead of 'CVDs' in the section “Strengths and limitations of this study”.</p>
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VERSION 2 – AUTHOR RESPONSE

REVIEWER 3: COMMENTS TO THE AUTHOR

I thank the authors for addressing my concerns in detail. The revisions to the manuscript have largely addressed my major concerns and improved the manuscript; only a few minor issues remain, which are addressed below:

1. Line 169-170: “Elastic-net analysis is expected to yield superior results...”. Have the authors checked the results for ridge, lasso and elastic-net regression separately to compare the results with each other?

Indeed, we have compared mean squared errors of LASSO, elastic-net and ridge regression models in a preliminary analysis. The Elastic-net model presented thereby the lowest error. As other studies also confirmed the expectation of superior results of the elastic-net models, compared to LASSO and ridge regression, especially when analysing highly correlated predictors [18, 19], we did not include the results of the preliminary analysis in the present paper.

As you have checked the results in a preliminary analysis, I would recommend adapting the wording of the sentence and avoiding the words "is expected to yield".

Thank you for your comment. We have adapted the wording as suggested.

"Elastic-net analysis has been shown to yield superior results, particularly in situations where predictors are highly correlated, as compared to LASSO and ridge regression [52, 53]." (line 183)

2. The authors emphasize the influence of sex hormones on arterial stiffness. Therefore, it might be worth adding a sentence in the discussion section focusing on arterial stiffness and menopause in women, considering a recently published paper by O`Neill et al. (2024) (<https://doi.org/10.1016/j.maturitas.2023.107900>).

Thank you for this interesting reference. We do agree that this should be mentioned in the discussion section:

"Prior studies have demonstrated the impact of sex hormones, such as oestrogens and testosterone, on arterial wall behaviour [21-23, 70]. Fluctuations in oestrogen levels seem to influence the impact of cardiovascular risk factors on PWV both across hormonal phases in women and in comparison to men [22, 23, 25]. A recent study identified a clinically significant acceleration in arterial stiffness during the late post-menopause phase (8+ years), probably related to a long-term exposition to the withdrawal of oestrogen, and potentially increasing the risk of CVD during this life stage [71]. Moreover, testosterone deficiency has been linked to increased PWV, indicative of early vascular aging [22]. The substantial impact of sex hormones on arterial stiffness underscores the importance of considering sex-specific factors in the assessment of cardiovascular health" (line 323-331).

3. Line 35: Please use 'cardiovascular diseases' instead of 'CVDs' in the section "Strengths and limitations of this study".

We adapted the corresponding sentence:

"Pulse Wave Velocity was used to assess arterial stiffness, which has been proven to be among the most important predictors of future cardiovascular diseases" (line 36-37).