

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)	The influence of shift work on cardiovascular disease risk in Southern African long-distance truck drivers: A cross-sectional study
AUTHORS	Draaijer, Melvin; Scheuermaier, Karine; Lalla-Edward, Samanta Tresha; Fischer, Alex; Grobbee, Diederick; Venter, Francois; Vos, Alinda

VERSION 1 – REVIEW

REVIEWER	Kervezee, Laura Leiden University Medical Center
REVIEW RETURNED	24-May-2021

GENERAL COMMENTS	<p>Draaijer et al. aim to investigate the effect of night shift work on cardiovascular disease risk in long-distance truck drivers in South Africa. This is a relevant topic from an occupational health perspective that has been the topic of investigation in prior studies. The authors performed a cross-sectional study in 607 male truck drivers, approximately half of which only worked day shifts and half of which worked both day and night shifts. Using various markers of cardiovascular disease risk (Framingham risk score, atherosclerotic cardiovascular disease risk algorithm, left ventricular hypertrophy, and carotid intima-media thickness), the authors find no effect of night shift work on cardiovascular disease risk in this population. The use of multiple different markers of cardiovascular disease risk is a strength of the study. However, besides the usual limitations related to a cross-sectional study design, I have several other comments about this work.</p> <p>Major comments:</p> <ol style="list-style-type: none">1) Introduction: to introduce what is known about night shifts and cardiovascular disease risk, the authors write that “exposure to shift work for 5 years has been associated with a 7% increased CVD risk [9]” (p. 5, line 30-31). To put the current study into perspective of the body of literature that is already available on this topic, it is important to mention in the introduction & discussion that the article that the authors cite to support this statement is a large systematic review and meta-analysis that was published in 2018, which combines the result from 21 cohort and case-control studies with in total 173 010 unique participants.2) Related to the previous comment, ref. 9 does not show that “exposure to shift work for 5 years has been associated with a 7% increased CVD risk” (p. 5, line 30-31) but that CVD risk increases with 7.1% for every five years of shift work exposure after the first five years. This should be adjusted in the text.3) Another meta-analysis on this topic was also published in 2018 (https://doi.org/10.1177/2047487318783892). In addition, a cross-sectional study on a similar topic in shift workers was published recently: https://doi.org/10.1186/s40557-017-0166-z (hypertension
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	<p>and shift work). Both studies should be cited and discussed in relation to the results of the present study.</p> <p>4) The authors report that they conducted sensitivity analyses using different cut-offs for night shift work and work history, but the results are not included in the manuscript. Given the previous findings that a dose-dependent relationship may exist, the manuscript would be stronger if they authors would include these results and statistics (e.g. as table(s) in the Appendix).</p> <p>5) Given the dose-dependent relationship reported by ref. 9, can the authors provide more information about the number of years that the workers had been working on the same schedule (i.e. day-night shifts or exclusively day shifts)? Did the authors use any cut-off with respect to the number of years involved in night shift work in their main analysis?</p> <p>6) Regarding the STROBE guidelines: how did the authors arrive at the sample size? What was the power to detect an effect with this sample size?</p> <p>7) A possible limitation of the cross-sectional study design is potential bias as a result of the healthy worker effect, by which workers that are relatively more fit continue to do night shifts, while others switch to a day shift or to a different job entirely. Did the authors consider this limitation? Please add to the discussion.</p> <p>Minor comments:</p> <p>1) Abstract, line 44: "a night shift was defined as working between 10pm and 6am" this definition should be described more precisely and in line with the methods used (e.g. a night shift was defined as working at least three hours once a week between 10pm and 6am)</p> <p>2) P. 8, line 55: what transformation(s) was/were done to meet criteria for normal distribution?</p> <p>3) P. 9, line 7: what was the rationale for including relationship status as a confounder?</p> <p>4) Table 1: n of HDL cholesterol is missing for subgroups</p>
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REVIEWER	Sweeney, Ellen Dalhousie University, Atlantic PATH
REVIEW RETURNED	15-Jun-2021

GENERAL COMMENTS	<p>This is an interesting paper focused on cardiovascular risk and shift work among truck drivers in Southern Africa. There is a growing body of evidence that shift work is associated with and/or influences the risk of CVD. There is clear justification for conducting this research and although the findings indicate that CVD risk did not differ between day shift and day-night shift, there was important evidence about CVD risk factors among both populations. An interesting addition to the literature. A few specific revisions/proofreading considerations below:</p> <p>Pg 3, Line 38 (Abstract) Consider revising to "sociodemographic, occupational and health characteristics" Pg 3, Line 40 Revise to "Physical measurements, an electrocardiogram (ECG), and carotid intima-media thickness (CIMT) measurements were taken" Pg 3, Line 52 Remove comma after "included" and before "of" Pg 4, Line 34 Revise to "With 607 participants, this is the largest cohort of male truck drivers in South Africa and to the best of our knowledge, the largest in Africa" Pg 5, Line 8 Consider adding "representing" before 31% Pg 5, Line 12 Typo, remove "is" from "making it is the second" Pg 6, Line 14 Consider revising to "HIV/AIDS" Pg 5, Line 18 Consider revising "lifestyle related conditions" to behavioural factors.</p>
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You also might consider removing the high BMI and replacing with a high waist to hip ratio to reflect the critiques of BMI as an independent indicator of health. Pg 5, Line 24 Consider adding "high levels of sedentary behaviour" in the list of reasons truck drivers are a high risk population for CVD. Pg 6, line 32 I would recommend adding additional references linking shift work to CVD as this is the primary focus of your article. In addition to Torquati et al., you might consider: Asare-Anane H, AbdulLatif A, Kwaku Ofori E, Abdul-Rahman M, Amanquah S (2015) Shift work and the risk of cardiovascular disease among workers in cocoa company. Tema. BMC Res Notes. <https://doi.org/10.1186/s13104-015-1750-3> Brum MCB, Filho FFD, Schnorr CC, Bottega GB, Rodrigues TC (2015) Shift work and its association with metabolic disorders. Diabetol Metabolic Syndrome 7:45 Gu F, Han J, Laden F, Pan A, Caporaso NE, Stampfer M et al (2015) Total and cause-specific mortality of US nurses working rotating night shifts. Am J Prevent Med 48(3):241–252. <https://doi.org/10.1016/j.amepre.2014.10.018> Pimenta AM, Kac G, Souza RR, Ferreira LM, Silqueira SM (2011) Night-shift work and cardiovascular risk among employees of a public university. Rev Assoc Med Bras 58(2):168 Sweeney E, Yu ZM, Dummer TJB, Cui Y, DeClercq V, Forbes C, Grandy SA, Keats M, Parker L, Adisesh A. (2019) The relationship between anthropometric measures and cardiometabolic health in shift work: findings from the Atlantic PATH Cohort Study. International Archives of Occupational and Environmental Health, 93(1) 67-76 doi: 10.1007/s00420-019-01459-8 Pg 5, paragraph 3. Move this entire paragraph above the previous one. Then have the two sentences about truck drives lead directly into the one sentence in the last paragraph. Pg 6, Line 13 "NSA provided health care services to truck drivers." For a reader who is not from South Africa, please provide more details on what the North-Star Alliance is. Furthermore, please provide additional details about the health care services this organization provides to truck drivers. Is it health care in general to support their wellbeing? Is this it the measurements that you took for this study? It's unclear. Pg 6, line 36 Are all truck drives in South Africa male or was this an inclusion/exclusion criteria? E.g., only males were included and females were excluded. Pg 6, line 47 Remove the comma after "recruitment to" and before "and conduct" Pg 7, line 8-10 Consider revising the "behavioural/health" description so that behaviours are listed separately from relevant health conditions. Also, add a comma after the "i.e." so it's consistent with your other examples in this sentence. Page 7, line 17 Revise "two to three night shift" to "two to three night shifts" Page 7 Add references for the Framingham Risk Score and the Atherosclerotic Cardiovascular Disease risk algorithm Page 7 You do measure the waist and hip circumference. Consider referencing the waist to hip ratio above instead of or in addition to BMI. Page 7, line 48 Re. the FRS calculated for participants without CVD. Did participants self-report CVD in a questionnaire? Pg 9, line 31. Can you please provide detail as to why the 607 participants had data which were included, but this is only 99% and not 100%? Pg 10, line 31 Revise "did also not" to "also did not" Pg 11, line 15 Are there any other reasons why your population might differ from those who have found an association between shift work and CVD? This is worth reflecting upon, as well as including more than one reference (#9) to literature that does support the finding. Pg 11, line 22 Double check that "14.000" is consistent with the journal's guidelines (rather than 14,000 or 14 000). Pg 11, line 28 Add a comma after "(.

	<p><30years)" and before "30%" Pg 11, line 35 Revise "was obese" to "were" or "are" Pg 11, line 38 Add "when compared to the general population" at the end of this sentence. Pg 12, line 3-4 Revise to "The combined LVH outcome may result in an overestimation of the number of participants without also conducting cardiac echocardiography which is considered the gold standard measure" Pg 12, line 16-17 Revise to "This is the largest cohort of male truck drivers in South Africa and to the best of our knowledge, the largest in Africa." Pg 12, line 17 Revise to "Our data represents..." Pg 12, line 23 You can state this strength with more emphasis. Add in some content about using the risk scores and physical measures, etc. Pg 18 -- Some of the references are mixed in with your tables. Perhaps a formatting error?</p>
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VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Dr. Laura Kervezee, Leiden University Medical Center

Comments to the Author:

Draaijer et al. aim to investigate the effect of night shift work on cardiovascular disease risk in long-distance truck drivers in South Africa. This is a relevant topic from an occupational health perspective that has been the topic of investigation in prior studies. The authors performed a cross-sectional study in 607 male truck drivers, approximately half of which only worked day shifts and half of which worked both day and night shifts. Using various markers of cardiovascular disease risk (Framingham risk score, atherosclerotic cardiovascular disease risk algorithm, left ventricular hypertrophy, and carotid intima-media thickness), the authors find no effect of night shift work on cardiovascular disease risk in this population. The use of multiple different markers of cardiovascular disease risk is a strength of the study. However, besides the usual limitations related to a cross-section study design, I have several other comments about this work.

Major comments:

1) Introduction: to introduce what is known about night shifts and cardiovascular disease risk, the authors write that “exposure to shift work for 5 years has been associated with a 7% increased CVD risk [9]” (p. 5, line 30-31). To put the current study into perspective of the body of literature that is already available on this topic, it is important to mention in the introduction & discussion that the article that the authors cite to support this statement is a large systematic review and meta-analysis that was published in 2018, which combines the result from 21 cohort and case-control studies with in total 173 010 unique participants.

Response: Thank you for this comment. We added the suggested information to the introduction of our paper.

Page 5, lines 11-15: “In a large systematic review and meta-analysis published in 2018, which combined the results from 21 cohort and case-control studies with a total of 173.010 unique participants, CVD risk increases with 7.1% for every five years of shift work exposure after the first five years^[7].”

2) Related to the previous comment, ref. 9 does not show that “exposure to shift work for 5 years has been associated with a 7% increased CVD risk” (p. 5, line 30-31) but that CVD risk increases with 7.1% for every five years of shift work exposure after the first five years. This should be adjusted in the text.

Response: Thank you for this comment. We adjusted the text as suggested (see sentence above).

3) Another meta-analysis on this topic was also published in 2018 (<https://doi.org/10.1177/2047487318783892>). In addition, a cross-sectional study on a similar topic in shift workers was published recently: <https://doi.org/10.1186/s40557-017-0166->

z (hypertension and shift work). Both studies should be cited and discussed in relation to the results of the present study.

Response: Thank you for these additional studies. We have included these studies in the discussion.

Page 11, lines 7-18: "However, other studies did find an increased CVD risk for night shift workers. In a systematic review and meta-analysis, shift work for more than five years had a positive and significant dose-response relationship on CVD risk. Shift work less than five years did not have a relation with CVD risk^[7]. Another study, also a systematic review and meta-analysis, demonstrated that an increase in shift work of five years was associated with a five percent increase in the risk of CVD^[36]. A third single site study with nearly 2000 participants showed that in male petrochemical plant workers, exposure to night shift work for over 20 years lead to a significant higher risk of getting hypertension^[37]. Our study lacked data on intension and duration of nightshifts so a dose-response relationship could not be investigated. Secondly, the group of truck drivers in our dataset who worked longer than 20 years was too small to do additional analysis."

4) The authors report that they conducted sensitivity analyses using different cut-offs for night shift work and work history, but the results are not included in the manuscript. Given the previous findings that a dose-dependent relationship may exist, the manuscript would be stronger if they authors would include these results and statistics (e.g. as table(s) in the Appendix).

Response: Thank you for this comment. We included these results in the appendix (Appendix 2, 3 and 4).

Page 9, lines 17-19: "In a sensitivity analysis, above described analyses were repeated using different cut-off points for night shift work, namely one night shift a week, two to three night shifts a week or more than four night shifts a week."

Page 10, lines 18-21: "Repeating the analysis using different definitions for night shift work resulted in the same findings (Appendix 2-3). Limiting the analysis to truck drivers who had been working as a truck driver for more than 10 years (n=229) did also not show a difference in CVD outcomes between day and day/night shift workers (Appendix 4)."

5) Given the dose-dependent relationship reported by ref. 9, can the authors provide more information about the number of years that the workers had been working on the same schedule (i.e. day-night shifts or exclusively day shifts)? Did the authors use any cut-off with respect to the number of years involved in night shift work in their main analysis?

Response: We agree that the mentioned dose-dependent relationship would be interesting to apply and research in our population. Unfortunately we do not have information on the number of years that drivers have been working in night shifts. We only have data available on the number of years they have been driving, and if they are working in day or night shifts. We do not have information on the duration of night shift work. We analyzed the impact of time in a sensitivity analysis including only drivers who had been working >10 years. We realize though that this does not mean necessarily that they were working in shifts all through. This has been addressed in the limitation section, page 12, lines 3-9:

'Some limitations need to be mentioned. The first relates to our definition of night shifts, as only 3 hours of work between 10pm and 6am classified someone as a night shift worker. To account for this, we did additional sensitivity analyses using different cut-offs for the number of nights worked in a week. Unfortunately we did not have information on the exact number of hours worked per night nor did we have information on the time a driver had been involved in shiftwork. This limits our analysis on the dose-response relationship between shiftwork and CVD risk.'

6) Regarding the STROBE guidelines: how did the authors arrive at the sample size? What was the power to detect an effect with this sample size?

Response: The trucker health survey aimed to get insight into health hazards of long distance truck drivers. Given the broad objective of the study no sample size calculation was performed, but as

many drivers as possible within the given timeframe were enrolled. Our study is a secondary data analysis and no sample size calculation was performed. This has been accounted for by adding the following sentence to the methods section:

Page 6, line 5: 'This analysis is a secondary data analysis of The Trucker Health Survey (THS).'

7) A possible limitation of the cross-sectional study design is potential bias as a result of the healthy worker effect, by which workers that are relatively more fit continue to do night shifts, while others switch to a day shift or to a different job entirely. Did the authors consider this limitation? Please add to the discussion.

Response: Thank you for this helpful comment. We agree that it may lead to an underestimation of the CVD risk in the night shift group. We added this to the discussion as followed.

Page 12, lines 10-15: "Another limitation is potential bias due to the healthy worker effect. Workers who are relatively fitter might do night shifts more often and will continue to do night shifts for a longer period of time. More unhealthy workers might possibly switch to day shifts only or to a different job. Although CVD risk factors did not differ between day and night shift workers there might be unmeasured risk factors leading to an underestimation of the influence of night shift work on CVD risk.

Minor comments:

1) Abstract, line 44: "a night shift was defined as working between 10pm and 6am" this definition should be described more precisely and in line with the methods used (e.g. a night shift was defined as working at least three hours once a week between 10pm and 6am)

Response: Thank you for this comment. We changed the definition in the abstract to a more precise definition.

Page 3, lines 19-20: "A night shift was defined as working at least 3 hours between 10pm and 6am once a week."

2) P. 8, line 55: what transformation(s) was/were done to meet criteria for normal distribution?

Response: Thank you for this comment. We added this information.

Page 9, lines 2-4: "Non-normally distributed data was transformed using the Box-Cox technique combined with a goodness of fit test using normal, lognormal and exponential distributions."

3) P. 9, line 7: what was the rationale for including relationship status as a confounder?

Response: We expected that relationship status could impact on the willingness to work night-shifts, and being in a stable relationship has been linked to a healthier lifestyle and hence CVD risk. We have included a reference to the methods.

Page 9, lines 10-11: "Variables considered as confounders for all outcomes were age, country of origin, education level and relationship status^[33]."

4) Table 1: n of HDL cholesterol is missing for subgroups

Response: Thank you for this comment and for noticing this error. We added the n for the subgroups (n = 296 and n = 291).

Reviewer: 2

Dr. Ellen Sweeney, Dalhousie University

Comments to the Author:

This is an interesting paper focused on cardiovascular risk and shift work among truck drivers in Southern Africa. There is a growing body of evidence that shift work is associated with and/or influences the risk of CVD. There is clear justification for conducting this research and although the findings indicate that CVD risk did not differ between day shift and day-night shift, there was important

evidence about CVD risk factors among both populations. An interesting addition to the literature. A few specific revisions/proofreading considerations below:

Comment 1:

Pg 3, Line 38 (Abstract) Consider revising to "sociodemographic, occupational and health characteristics"

Response: Thank you, we changed this sentence as suggested.

Page 3: "Information was collected on sociodemographics, occupational and health characteristics."

Comment 2:

Pg 3, Line 40 Revise to "Physical measurements, an electrocardiogram (ECG), and carotid intima-media thickness (CIMT) measurements were taken"

Response: Thank you, we changed this sentence as suggested.

Page 3: "Physical measurements, an electrocardiogram (ECG), and carotid intima-media thickness (CIMT) measurements were taken."

Comment 3:

Pg 3, Line 52 Remove comma after "included" and before "of"

Response: Thank you, we changed this sentence as suggested.

Page 3: "In total, 607 truck drivers were included of which 305 (50.2%) worked in day shifts only and 302 (49.8%) worked day and night shifts."

Comment 4:

Pg 4, Line 34 Revise to "With 607 participants, this is the largest cohort of male truck drivers in South Africa and to the best of our knowledge, the largest in Africa"

Response: Following the editors and your comment and the guidelines of the journal (85 characters maximal including spaces) we changed the sentence to:

Page 4: This study presents the largest cohort of male truck drivers in Africa.

Comment 5:

Pg 5, Line 8 Consider adding "representing" before 31%

Response: Thank you, we changed this sentence as suggested.

Page, line "An estimated 17.9 million people died of CVD in 2016, representing 31% of all global deaths."

Comment 6:

Pg 5, Line 12 Typo, remove "is" from "making it is the second"

Response: Thank you, we changed this sentence as suggested.

Page 5: "In South Africa, CVD is responsible for approximately 20% of all deaths, making it the second leading cause of death after HIV."

Comment 7:

Pg 6, Line 14 Consider revising to "HIV/AIDS"

Response: Thank you for this comment. We assumed that you referred to page 5 line 14. We incorporated your suggestion:

Page 6 line: "In South Africa, CVD is responsible for approximately 20% of all deaths, making it the second leading cause of death after HIV/AIDS."

Comment 8:

Pg 5, Line 18 Consider revising "lifestyle related conditions" to behavioural factors. You also might consider removing the high BMI and replacing with a high waist to hip ratio to reflect the critiques of BMI as an independent indicator of health.

Response: Thank you for this comment. High cholesterol, high blood pressure and high body mass index are lifestyle related conditions but in our opinion not necessarily only behavioral factors as it might also be the environment (like food choices available, quality of the environment). Regarding your second comment we agree that waist to hip ratio is an independent factor of health and more accurate than BMI. However, as we included BMI as a variable we decided to mention both. The sentence now reads as followed.

Page 5: "The cause of CVD is multifactorial and includes behavioral factors such as smoking, physical inactivity, unhealthy dietary patterns and lifestyle related conditions such as high cholesterol, high blood pressure, and high body mass index and high waist to hip ratio."

Comment 9:

Pg 5, Line 24 Consider adding "high levels of sedentary behaviour" in the list of reasons truck drivers are a high risk population for CVD.

Response: Thank you for this comment. We incorporated your suggestion. The sentence now reads as followed.

Page 5: "Truck drivers are a high risk population for CVD by virtue of their occupation with long working hours, frequent shift work, low physical activity and high levels of sedentary behaviour."

Comment 10:

Pg 6, line 32 I would recommend adding additional references linking shift work to CVD as this is the primary focus of your article. In addition to Torquati et al., you might consider: Asare-Anane H, AbdulLatif A, Kwaku Ofori E, Abdul-Rahman M, Amanquah S (2015) Shift work and the risk of cardiovascular disease among workers in cocoa company. Tema. BMC Res Notes. <https://doi.org/10.1186/s13104-015-1750-3>. Brum MCB, Filho FFD, Schnorr CC, Bottega GB, Rodrigues TC (2015) Shift work and its association with metabolic disorders. Diabetol Metabolic Syndrome 7:45 Gu F, Han J, Laden F, Pan A, Caporaso NE, Stampfer M et al (2015) Total and cause-specific mortality of US nurses working rotating night shifts. Am J Prevent Med 48(3):241–252. <https://doi.org/10.1016/j.amepre.2014.10.018> Pimenta AM, Kac G, Souza RR, Ferreira LM, Silqueira SM (2011) Night-shift work and cardiovascular risk among employees of a public university. Rev Assoc Med Bras 58(2):168 Sweeney E, Yu ZM, Dummer TJB, Cui Y, DeClercq V, Forbes C, Grandy SA, Keats M, Parker L, Adishes A. (2019) The relationship between anthropometric measures and cardiometabolic health in shift work: findings from the Atlantic PATH Cohort Study. International Archives of Occupational and Environmental Health, 93(1) 67-76 doi: 10.1007/s00420-019-01459-8

Response: Thank you for this comment. We added the interesting article on cocoa workers in Ghana. In line with a comment from the second reviewer we added references in the discussion to further elaborate on linking shift work to CVD.

Comment 11:

Pg 5, paragraph 3. Move this entire paragraph above the previous one. Then have the two sentences about truck drives lead directly into the one sentence in the last paragraph.

Response: Thank you for this comment. After making the suggested change the introduction is easier to read.

Comment 12:

Pg 6, Line 13 "NSA provided health care services to truck drivers." For a reader who is not from South Africa, please provide more details on what the North-Star Alliance is. Furthermore, please provide additional details about the health care services this organization provides to truck drivers. Is it health care in general to support their wellbeing? Is this it the measurements that you took for this study? It's unclear.

Response: We added the following sentence.

Page 7: 'NSA provided health care services to truck drivers through a network of Roadside Wellness Centres located at busy truck stops and at border crossings^[12].'

Reference 12 is added to the sentence as in the referred paper more information on NSA is provided.

Comment 13:

Pg 6, line 36 Are all truck drives in South Africa male or was this an inclusion/exclusion criteria? E.g., only males were included and females were excluded.

Response: thanks for this interesting question. The vast majority of truck drivers are male, female drivers were estimated to be <5% when the THS was designed. As the group of women was estimated to be too small to draw any conclusions inclusion was limited to males. To inform the reader better on the methods of the Trucker Health Survey we included a reference to the methods paper:

Page 7 'Methods and characteristics of the THS have been described previously.'

Comment 14:

Pg 6, line 47 Remove the comma after "recruitment to" and before "and conduct"

Response: We changed the manuscript as suggested, thank you.

Comment 15:

Pg 7, line 8-10 Consider revising the "behavioural/health" description so that behaviours are listed separately from relevant health conditions. Also, add a comma after the "i.e." so it's consistent with your other examples in this sentence.

Response: we changed the manuscript accordingly. The sentences now read as followed.

Page 7: "Information on socio-demographic (i.e., age, education, country of origin, marital status), occupational (i.e., time spent working, working night shifts), behavioral (i.e., smoking status, physical activity, sleep duration per day) and health (i.e., HIV status, diabetes treatment, hypertension treatment) characteristics were collected using validated questionnaires.

Comment 16:

Page 7, line 17 Revise "two to three night shift" to "two to three night shifts"

Response: Thank you for this correction. We changed is as suggested.

Comment 17:

Page 7 Add references for the Framingham Risk Score and the Atherosclerotic Cardiovascular Disease risk algorithm

Response: We added the references as suggested.

Comment 18:

Page 7 You do measure the waist and hip circumference. Consider referencing the waist to hip ratio above instead of or in addition to BMI.

Response: Thank you for this comment. This comment is a follow-up of the earlier provided comment #8. We added the waist to hip ratio in addition to BMI.

Comment 19:

Page 7, line 48 Re. the FRS calculated for participants without CVD. Did participants self-report CVD in a questionnaire?

Response: Thank you for your comment. In our survey participants reported on CVD although very few participant did report a CVD event, n = 3 reported to have had a cardiovascular event. We decided to remove part of this sentence. The sentence now reads as followed.

Page 7: "CVD risk according to the FRS was calculated and categorized in low-, intermediate- and high-CVD risk."

Comment 20:

Pg 9, line 31. Can you please provide detail as to why the 607 participants had data which were included, but this is only 99% and not 100%?

Response: Thank you for this question. The original database of participants included 614 truck drivers of which only 607 have data on shift work. These 7 truck drivers did not fill in the night shift work questionnaire/left it blank. 607/614 is 98.8%. To make this more clear we changed the first sentence of the results.

Page 9: "In total, 614 male truck drivers completed the survey, of which 607 (99%) had data on shift work available. Nearly half (n=305, 50.2%) worked in day shifts only and 302 drivers (49.8%) worked both day and night shifts (Table 1)."

Comment 21:

Pg 10, line 31 Revise "did also not" to "also did not"

Response: Thank you for this correction. We changed the manuscript as suggested.

Page 10: "Limiting the analysis to truck drivers who had been working as a truck driver for more than 10 years (n=229) also did not show a difference in CVD outcomes between day and day/night shift workers."

Comment 22:

Pg 11, line 15 Are there any other reasons why your population might differ from those who have found an association between shift work and CVD? This is worth reflecting upon, as well as including more than one reference (#9) to literature that does support the finding.

Response: your comment is in line with a comment from the other reviewer. We decided to change the discussion to include more references that did find an association between shift work and CVD. The discussion now reads as followed.

Page 11: "However, other studies did find an increased CVD risk for night shift workers. In a systematic review and meta-analysis shift work for more than five years had a positive and significant dose-response relationship on CVD risk. Shift work less than five years did not have a relation with CVD risk^[7]. Another study, also a systematic review and meta-analysis, demonstrated that an increase in shift work of five years was associated with a five percent increase in the risk of CVD^[36]. A third single site study with nearly 2000 participants showed that in male petrochemical plant workers, exposure to night shift work for over 20 years lead to a significant higher risk of getting hypertension^[37]. Our study lacked data on intension and duratioof nightshifts so a dose-response relationship could not be investigated. Secondly, the group of truck drivers in our dataset who worked longer than 20 years was too small to do additional analysis.

Comment 23:

Pg 11, line 22 Double check that "14.000" is consistent with the journal's guidelines (rather than 14,000 or 14 000).

Response: Thank you. The current method is consistent with the journal's guidelines.

Comment 24:

Pg 11, line 28 Add a comma after "(. <30years)" and before "30%"

Response: Thank you for this correction. We changed the manuscript as suggested.

Comment 25:
Pg 11, line 35 Revise "was obese" to "were" or "are"

Response: Thank you for this correction. We changed the manuscript as suggested.

Comment 26:
Pg 11, line 38 Add "when compared to the general population" at the end of this sentence.

Response: Thank you for this correction. We changed the manuscript as suggested.

Comment 27:
Pg 12, line 3-4 Revise to "The combined LVH outcome may result in an overestimation of the number of participants without also conducting cardiac echocardiography which is considered the gold standard measure"

Response: Thank you for this correction. We changed the manuscript as suggested.

Comment 28:
Pg 12, line 16-17 Revise to "This is the largest cohort of male truck drivers in South Africa and to the best of our knowledge, the largest in Africa."

Response: Thank you for this comment. We changed the manuscript as suggested.

Comment 29:
Pg 12, line 17 Revise to "Our data represents..."

Response: Thank you for this revision. We changed the manuscript accordingly.

Comment 30:
Pg 12, line 23 You can state this strength with more emphasis. Add in some content about using the risk scores and physical measures, etc.

Response: Thank you for this comment. The sentence now reads as followed.

Page 12: "Another strength is that we defined CVD risk in complementary ways using with four different outcome measures namely FRS, ASCVD, LVH on ECG and CIMT in combination with the wide variety of physical measurements."

Comment 31
Pg 18 -- Some of the references are mixed in with your tables. Perhaps a formatting error?

Response: Apologies for the inconvenience. In the original copy of the manuscript this was not the case. The current manuscript does not show any formatting errors.

VERSION 2 – REVIEW

REVIEWER	Kervezee, Laura Leiden University Medical Center
REVIEW RETURNED	11-Oct-2021
GENERAL COMMENTS	The authors have addressed most of my comments. My remaining concern is that the authors have now clarified that they have no information available regarding the number of years that the participants had been working night shifts and that no cutoff

	<p>regarding number of years of exposure was used to classify workers as night workers. I feel this is crucial information given the relationship between years of exposure to night shifts and CVD risk that is reported in meta-analyses. The need to include this in shift work studies is also described in a paper on the definition of shift work in epidemiological studies by the IARC working group (https://oem.bmj.com/content/68/2/154.short). In prior studies that reported negative results this information was also available and included in the statistical analyses (e.g. ref. 34 and 35), so I am not suggesting that the results may have been different if this information were available, but my concern is that the absence of this information makes the results less convincing.</p> <p>Other comments:</p> <ul style="list-style-type: none"> - Could the authors add an explanation what the p-values in Table 2 represent and why these are different from the p-values in Appendix 1 in the table rows showing the (multivariable) effect of day/night shift? I missed this last time, but it is not clear to me what test these p-values were derived from. - I commend the authors for including the sensitivity analysis based on number of night shifts per week. It is important to include the sample size that these analyses were based on. Do the authors think they would have had more power to detect an effect if they would have used a model with number of night shifts per week as a variable (as in previous literature)? What the reason is for performing these separate analyses?
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VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

Dr. Laura Kervezee, Leiden University Medical Center

Comment 1:

The authors have addressed most of my comments. My remaining concern is that the authors have now clarified that they have no information available regarding the number of years that the participants had been working night shifts and that no cutoff regarding number of years of exposure was used to classify workers as night workers. I feel this is crucial information given the relationship between years of exposure to night shifts and CVD risk that is reported in meta-analyses. The need to include this in shift work studies is also described in a paper on the definition of shift work in epidemiological studies by the IARC working group (<https://oem.bmj.com/content/68/2/154.short>). In prior studies that reported negative results this information was also available and included in the statistical analyses (e.g. ref. 34 and 35), so I am not suggesting that the results may have been different if this information were available, but my concern is that the absence of this information makes the results less convincing.

Response:

We fully agree with the reviewer that information on the duration of shift work would have added valuable information. We acknowledge this lack of information in the discussion in the following sentences:

Page 11 line 15-18: 'Our study lacked data on intension and duration of nightshifts so a dose-response relationship could not be investigated. Secondly, the group of truck drivers in our dataset who worked longer than 20 years was too small to do additional analysis.'

Page 12 lines 6-9: 'Unfortunately we did not have information on the exact number of hours worked

per night nor did we have information on the time a driver had been involved in shiftwork. This limits our analysis on the dose-response relationship between shiftwork and CVD risk.'

Comment 2:

Could the authors add an explanation what the p-values in Table 2 represent and why these are different from the p-values in Appendix 1 in the table rows showing the (multivariable) effect of day/night shift? I missed this last time, but it is not clear to me what test these p-values were derived from.

Response:

Thank you for this comment. We first explored differences in the cardiovascular risk parameters between day and night shift workers using a Chi-square test for categorical outcomes and a Mann-Whitney-U test for continuous (all non-normally distributed) outcomes. Results of this analysis are presented in table 2.

This has been clarified in the statistics section in the following sentence:

Page 9, lines 4-7: 'To test how cardiovascular measures differed between day and night shift workers a Chi-square test was used for categorical outcomes and a Mann-Whitney-U test was used for continuous outcomes.'

To improve the understanding of the results we merged the description of this analysis to one paragraph. It now reads as follows: page 10, lines 6-11: 'Shift work was borderline associated with a difference in FRS ($p = 0.05$) as continuous outcome, but there was no difference between the groups when categorized in low, intermediate and high risk ($p = 0.57$). Shift work was not associated with ASCVD risk score ($p = 0.94$), LVH occurrence (all $p > 0.20$) or CIMT, except for max bulb IMT, which was higher in day shift workers compared to day-night shift workers ($p < 0.01$) (Table 2).'

Finally, we amended the heading of table to from 'Descriptive statistics of cardiovascular risk assessments' to 'Cardiovascular risk assessments between dayshift only and day-night shift drivers.'

Second, we analysed the influence of shift work on the cardiovascular parameters in a regression analysis.

This has also been clarified in the statistical analysis part:

Page 9 lines 7-15: 'Next, regression analysis was used to assess the influence of shift work on FRS, ASCVD risk, mean CCA-IMT and LVH while adjusting for confounders. Variables considered as confounders were age, country of origin, education level and relationship status[33]. We did not adjust for known CVD risk factors as outcomes represent the cumulative effect of CVD risk factors. Variables were included in multivariable analysis if the p-value was ≤ 0.20 in univariable analysis. Age was added to the multivariable model independent of the p-value in univariable analysis. FRS, ASCVD and mean CCA-IMT were log transformed to meet criteria for normal distribution.'

The results section has also been updated to increase clarity:

Page 10, lines 12-13: 'Following multivariable regression analysis shift work was not associated with any of the cardiovascular outcomes.'

Comment 3:

I commend the authors for including the sensitivity analysis based on number of night shifts per week. It is important to include the sample size that these analyses were based on. Do the authors think they would have had more power to detect an effect if they would have used a model with number of night shifts per week as a variable (as in previous literature)? What the reason is for performing these

separate analyses?

Response:

Thank you for this comment. We added the sample sizes to appendix 2 (n=228), appendix 3 (n=74) and appendix 4 (n=229).

Number of night shifts per week was collected in three categories: 0-1 night shifts a week, 2-3 night shifts a week or ≥ 4 night shifts a week. Hence, we could not use night shifts a week as a continuous outcome. In the sensitivity analysis we maximized the contrast between the groups by comparing the group with 2-3 night shift a week to 0-1 shifts a week, and ≥ 4 night shifts a week to 0-1 shifts a week. We did not find any difference between the intensity of night shifts and CVD risk in this analysis, so we don't expect that we would have found a dose-response relationship if we would have had number of night shifts a week as a continuous variable.

VERSION 3 – REVIEW

REVIEWER	Kervezee, Laura Leiden University Medical Center
REVIEW RETURNED	20-Jan-2022
GENERAL COMMENTS	I have no further comments.