

Author's response to reviews

Title: Heart rate variability among Nunavik Inuit adults exposed to environmental mercury: a cross-sectional study.

Authors:

Beatriz Valera MSc (beatriz.valera@crchul.ulaval.ca)
Eric Dewailly MD PhD (eric.dewailly@crchul.ulaval.ca)
Paul Poirier MD PhD (paul.poirier@crhl.ulaval.ca)

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Author's response to reviews:

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Professor Philippe Grandjean, MD, DMSc
Editor-in-Chief, Environmental Health
University of Southern Denmark
Odense 5000 Denmark

Professor David Ozonoff, MD
Editor-in-Chief, Environmental Health
Boston University School of Public Health
Environmental Health
715 Albany Street
Boston MA 02118
United States

OBJECT: Manuscript 1071614343162017 version 2. Heart rate variability among Nunavik Inuit adults exposed to environmental mercury: a cross-sectional study.

Dear editors,

We would like to thank you for providing us with the opportunity to submit a revised version of our manuscript entitled "Heart rate variability among Nunavik Inuit adults exposed to environmental mercury: a cross-sectional study". We would also like to thank the reviewers for their thoughtful comments and questions regarding our manuscript. Please find below our detailed response to each of the reviewers' comments and questions as well as relevant references when appropriate.

We hope that you find our paper of interest to readers of Environmental Health. To the best of our knowledge, this study is the first to assess the influence of

mercury on heart rate variability in adults and we consider that the results could be important for professionals interested in environmental contaminants and human health.

Sincerely yours,

For the authors,

Valera B, MSc, PhD (cand.). Public Health Unit Research, Centre Hospitalier Universitaire de Québec, Université Laval, Québec, Canada

AUTHORS CONTRIBUTION

BV was involved in data analysis, interpretation of the results and in drafting the manuscript. ED is the principal investigator and has participated in the conception and design of the study, data collection and interpretation of the results. PP has participated in the conception and design of the study as well as in the interpretation of the results. All authors read and approved the final manuscript.

ANSWERS TO REVIEWERS

Manuscript 1071614343162017 version 2. Heart rate variability among Nunavik Inuit adults exposed to environmental mercury: a cross-sectional study.

COMMENTS FROM EDITORS

We would like to know if blood pressure is available. It seems from your response that subjects with increased blood pressure were excluded, but blood pressure could be an additional outcome variable that deserves to be considered.

We agree with the editors. In fact, blood pressure was measured in adults 18 years and older (n= 866). However, taking into account that data on blood pressure involve a greater number of individuals than that on HRV, we decided to present the impact of mercury on blood pressure in another manuscript that is part of my PhD thesis. Also, the fact of not including blood pressure does not affect the interpretation of the results obtained in the present study.

In the abstract, please indicate how mercury exposure was assessed. Also, the strongly significant association with LF can hardly be called 'weak'. Please rephrase. The conclusion needs to be reworded to indicate that your findings seem to be in accordance with previous observations, although they were not statistically significant.

Mercury exposure was assessed using blood mercury concentrations. The following statement was added in the abstract «Blood mercury concentration was used as exposure biomarker». The association with LF has been rephrased in the revised manuscript. We have written «Mercury was negatively correlated with low frequency». The conclusion was reworded as suggested by the editor and taking into account the new findings. The following statement was changed in the Conclusion section of the abstract «The results suggest a deleterious impact of

mercury on HRV in adults. Although only the association with SDANN was statistically significant, most of the regression coefficients were in the same direction than those reported in children. Taking into account that this is the first study in adults, the results obtained here must be confirmed in other populations. Also, further studies will also be carried out to investigate the role of n-3 fatty acids on HRV among Nunavik Inuit adults».

The regression coefficients given in the text are difficult to evaluate. It would be useful if you could compare these results with findings in other studies. Do your observations differ from those previously published? The reference to selenium as a protective factor must take into account that any effect has not been demonstrated in methylmercury-exposed humans so far. If selenium acts as a confounder, it could well be because of shared variance due to common origin with methylmercury, and adjustment would then be erroneous. Does it make a difference if selenium is not included in the models?

We did not compare our results with those from previous studies because there is no report in adults. We could compare regression coefficients with those obtained in Faroe Island by considering mercury blood concentrations at 7 and 14 years old as biomarker of current exposure. However, in Faroe Island, mercury concentrations at 7 and 14 years old were correlated with mercury concentrations in cord blood which did not allow to separate postnatal from prenatal exposure. In the revised version of the manuscript, we discussed our results with studies in children.

Regarding selenium, we agree with the editors that sources of mercury and selenium are the same and that no effect of selenium on heart rate variability has been reported. However, we included selenium as a potential confounder since previous studies have suggested that selenium can interact with mercury to diminish its toxicity. For example, a study suggested that selenoproteins played important roles in protecting against Hg toxicity [1]. Following the suggestions of the editors, we performed new statistical analyses excluding selenium as a potential confounder and the SDANN regression coefficient became significant in multivariable analysis. We also calculated CVRR in order to compare with results obtained in the Faroe Island. The association with CVRR was not statistically significant in multivariable analysis but the regression coefficient was in the same direction than that observed in Faroe Island and p-value approached the significance level (adjusted beta= -0.057, p= 0.056). Thus, we decided to exclude selenium as a potential confounder in the revised version of the manuscript since no influence on HRV has been documented and no protective effect has been observed in mercury-exposed populations.

The statement at the beginning of the first full paragraph on page 14 needs to take into account the confidence interval and the likely problem of imprecise exposure assessment. As it stands now, it seems to indicate that any exposure to mercury in adults has no impact at all on HRV. Such conclusion would be a great exaggeration. It is repeated in the Conclusions on the following page, which also needs revision.

As suggested, statements were rephrased in the revised version of the manuscript taking into account the new interpretation of data. Conclusions were rephrased as: «The results obtained in the present study suggest a deleterious impact of mercury on HRV in adults. Although only the association with SDANN was statistically significant, most of the regression coefficients were in the same direction than those observed in children. Taking into account that this is the first study in adults, the results obtained here must be confirmed in other populations. Also, further studies will also be carried out to investigate the role of n-3 fatty acids on HRV among Nunavik Inuit adults».

The title should reflect the study type. Capitalize only the first word, and proper nouns, in the title. Indicate full institutional addresses for all authors. The abstract should not be included in the title page. Pluralize Conclusion title (conclusions) in abstract. Repeating the title is not necessary. In References, use of et al is not appropriate, remove issue numbers. In references 4 & 6, format used is unclear (all should not be bolded for 4, missing information in 6), please modify. Reference 29 requires modification (unclear format, repeated information), perhaps use link format. Ref 31, modify format (author names should not be bolded, use Edited by instead of eds, etc.). Reference 32 is missing the volume number. In reference 33, if using institutional author format, title should not be bolded.

We made changes that editors suggested. References were revised and most of them modified. However, for reference 6 we do not have more information because this is a report from the Quebec National Institute of Public Health. Another reference was added for reference 29 in the revised version and reference 33 was not included in the revised version of the manuscript.

COMMENTS FROM REVIEWERS

Reviewer 1: Denise Felber Dietrich

Minor essential revisions

1) p. 2 line 12: Please change "multiple regression" also into "multivariable regression"

Done

2) p. 12 ff: I would prefer the results of the sensitivity analyses to be in the results section.

Sensitivity analyses were included in the Results section of the revised manuscript.

3) Table 1: Please define levels of physical activity explicitly

As suggested, physical activity was explicitly defined in Table 1 as well as in Methodology section.

Discretionary Revisions

1) Sensitivity Analyses: Tables of the results of the sensitivity analyses would be

helpful for the readers.

We agree with the reviewer. However, we decided not to include tables of the results of the sensitivity analyses because we feel that this information will decrease the readability of the tables. However, this information is included in the Results section of the revised manuscript.

Reviewer 4: Esben Budtz-Jorgensen

Sampling:

On page 5 it is stated: 'A proportional stratification by community was used as sampling method'. What are these communities? Were the 14 villages divided into a number of communities? Or is community the same as village? It seems that a two-stage sampling method was used. In the first step a stratified random sample of households were generated such the probability of selecting a household from a given community was proportional to the community number of households. The text should be more clear. In addition, on page 5, it is stated that 'the survey included all persons'. I guess this means that the target population was all 'all persons' but not all persons were included in the sample.

On page 5, the sampling method was explicitly explained through the paragraph below. Also, the statement 'the survey included all permanent residents' was changed by «the target population included all permanent residents».

The target population included all permanent residents, except for non-Inuit households and individuals living full time in public institutions. The survey plan was a complex two-stage stratified random sampling. The first stage was to select a stratified random sample of private Inuit households with proportional allocation. The community was the only stratification variable used. This stratification allowed the representation of the target population to be up to standard. Since home addresses (civic numbers) in some municipalities are consecutive, the survey frame was sorted first by home addresses, followed by a systematic draw of a predetermined number of households to avoid selection of two immediate neighbours. Since many Inuit regularly move from one house to another, it was decided to sample households instead of individuals. The assumption was that recruiting a member of a household rather than a specific individual, would increase coverage of the target population. To obtain a good representation of each community, a proportional allocation of sample units corresponding to the size of each village was chosen. It was important to choose households from all 14 communities since the distances separating villages could be associated with significant differences in lifestyle. In the second stage, all eligible people were asked to participate according to the survey steps or instruments. A total of 677 Inuit households were randomly selected. A total of 1527 individuals of 15 years and older were contacted. In the present study, we used the 2-hour Holter data collected only among 280 adults 40 years and older.

Bootstrap method

From the references it is clear that this is an appropriate method. However, the text is still unreadable. This method was only used to estimate the variance in

different estimators. This should be emphasized. The first sentence 'all variables were weighted using the bootstrap method' only holds for calculation of the variance in estimators. In contrast, when the parameters were estimated so-called sampling weights may have been used, this should be clarified but these weights would not have been calculated using the bootstrap. Instead these should be calculated to reflect the sampling design used in the survey.

It is not quite clear whether the bootstrap method was used also to determine variances in regression coefficients as I think it should. If the method was used throughout, I think it would be better to have the description in the end of 'Statistical Analysis'. At that point it will be clear to the reader which estimators are being considered, and then it would be logical to end the section by explaining how the variances in these estimators were calculated. Furthermore, given the complexity of the bootstrap method and the fact that appropriate references have now been included, I think that a shorter description would be better i.e. without mentioning the bootstrap weights. It should be stated how to obtain the Methodological report by Rochette and Blanchet.

Taking into account the comments of the reviewer, corrections were made to the explanation of the bootstrap method in the 'Methodology section'. In this study, bootstrap method was used to calculate the variance in means and proportions as well as in regression coefficients while for the calculation of these parameters sample weights were used. This was emphasized in the revised version of the manuscript. Paragraphs below were added to the Methodology section:

Taking into account the complex sampling design used in this study, sampling weights were incorporated into the calculation of descriptive statistics as well as linear regression parameters. Weighting participants answers takes into account the probability of selecting each individual as induced by the design of the survey, the rates of non-response and differences observed between the sample and the population. The weight corresponds to the number of persons in the entire population who are represented by the respondent. Thus, the estimates generated by using sampling weights could be generalized to the entire population of Nunavik. Details on methodology are available in the methodological report of the survey [2].

The paragraph below was added to the end of the statistical section as suggested by the reviewer:

Owing to the complex sampling method used in this study, the bootstrap technique was selected to determine variances in the descriptive statistics as well as the regression coefficients derived from the sampling design [2-4]. This technique provides precision measurements for estimates obtained from a complex sample design. The bootstrap is in fact a re-sampling method that consists of drawing subsamples (500 subsamples were used in this study) from the original sample and generating estimates for each of those subsamples. An estimation of the sampling variance is deduced by measuring the dispersion between those estimations. In order to extract an estimate for each subsample that could be inferred to the entire population, sample weighting must be used. It

involves the production of a set of weights for each subsample. These sets of weights are called bootstrap weights. The calculation of sampling weights as well as bootstrap weights for this survey was done by the Institut de la statistique du Québec. Details on methodology is available in the methodological report of the survey [2].

The methodological report by Rochette and Blanchet is available in electronic format (PDF) on the Institut national de santé publique du Québec. Web site <http://www.inspq.qc.ca/pdf/publications/nunavik.asp>

REFERENCES

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