HHS Public Access

Author manuscript

Environ Int. Author manuscript; available in PMC 2019 August 01.

Published in final edited form as:

Environ Int. 2018 August ; 117: 125–131. doi:10.1016/j.envint.2018.05.001.

Serum Mercury Concentration and the Risk of Ischemic Stroke: the REasons for Geographic and Racial Differences in Stroke Trace Element Study

Cheng Chen[a], **Pengcheng Xun**[a], **Leslie A. McClure**[b], **John Brockman**[c], **Leslie MacDonald**[d], **Mary Cushman**[e], **Jianwen Cai**[f], **Lisa Kamendulis**[g], **Jason Mackey**[h], and **Ka He**[a,*]

[a]Department of Epidemiology and Biostatistics, School of Public Health -- Bloomington, Indiana University, Bloomington, IN 47405, USA

[b]Department of Epidemiology and Biostatistics, Dornsife School of Public Health, Drexel University, Philadelphia, PA 19104, USA

[c]Columbia Research Reactor, University of Missouri, Columbia, MO 65211, USA

[d]National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Cincinnati, OH 45226, USA

[e]Department of Medicine, Larner College of Medicine at the University of Vermont, Burlington, VT 05401, USA

[f]Department of Biostatistics, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

[g]Department of Environmental Health, School of Public Health -- Bloomington, Indiana University, Bloomington, IN 47405, USA

[h]Department of Neurology, School of Medicine, Indiana University, Indianapolis, IN 46202, USA

Abstract

Background—Although biologically plausible, epidemiological evidence linking exposure to methylmercury with increased risk of ischemic stroke is limited. The effects of methylmercury may be modified by selenium, which is an anti-oxidant that often co-exists with mercury in fish.

[*]Corresponding author: Dr. Ka He, Department of Epidemiology and Biostatistics, School of Public Health -- Bloomington, Indiana University, 1025 E. Seventh Street, SPH C042, Bloomington, Indiana 47405, USA (Phone: 812-856-2260; kahe@indiana.edu).

Declarations of interest

None.

Disclaimer

The content of this paper is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Neurological Disorders and Stroke, the National Institutes of Health, or the National Institute for Occupational Safety and Health.

Objectives—To examine the association between serum mercury levels with the incidence of ischemic stroke and to explore the possible effect modifications by serum selenium levels and demographic and geographic factors.

Methods—A case-cohort study was designed nested in the REasons for Geographic and Racial Differences in Stroke cohort, including 662 adjudicated incident cases of ischemic stroke and 2,494 participants in a randomly selected sub-cohort. Serum mercury was measured using samples collected at recruitment. Multivariable-adjusted hazard ratios (HRs) and the corresponding 95% confidence intervals (CIs) were estimated using the Barlow-weighting method for the Cox proportional hazards regression model.

Results—No statistically significant association was observed between serum mercury concentration and the incidence of ischemic stroke (the highest *vs.* lowest quintile of mercury levels: HR=0.82; 95% CI=0.55–1.22; *P* for linear trend=0.42). Sex (*P* for interaction=0.06), but not serum selenium levels, modified the association; a more evident trend toward lower incidence of ischemic stroke with higher mercury levels was observed among women.

Conclusion—This study does not support an association between mercury and the incidence of ischemic stroke within a population with low-to-moderate level of exposure. Further studies are needed to explore the possibility of mercury-induced ischemic stroke toxicity in other populations at higher exposure levels.

Keywords

mercury; selenium; ischemic stroke; case-cohort study; REGARDS study

1. Introduction

Stroke accounts for approximately 5% of all deaths in the U.S. (National Center for Health Statistics, 2011). Despite great efforts to identify risk factors and interventions focusing on modifying unhealthy lifestyles (Lackland et al., 2014), higher stroke incidence and mortality are observed in women, blacks and residents of the “Stroke Belt” region (Lanska, 1993). To try to explain these disparities, the importance of environmental factors in relation to stroke risk has been increasingly recognized.

Mercury is one of the most toxic heavy metals (Solenkova et al., 2014). Methylmercury from fish and other seafood is a major environmental source of potential mercury toxicity (Guallar et al., 2002), which has raised extensive concerns since fish consumption is recommended to the public for its cardio-protective effects due to long-chain n-3 polyunsaturated fatty acids (LCn3PUFAs). Existing studies have suggested some basic mechanisms by which mercury could increase the risk of stroke, and epidemiological evidence links mercury exposure to hypertension (Houston, 2011). However, data are limited regarding the effect of mercury exposure on stroke risk. Previous studies have been inconclusive and failed to observe an association (Daneshmand et al., 2016; D. Mozaffarian et al., 2011; Wennberg et al., 2007).

In addition, selenium often co-exists with mercury in fish and is an antioxidant that may improve cardiovascular health (Hansen et al., 1994). The observation that selenium is an

antagonist of mercury toxicity is well demonstrated in animal studies (Ganther et al., 1972; Seppanen et al., 2000; Sumino et al., 1977). However, the possible interaction between selenium and mercury has not been adequately studied in humans (D. Mozaffarian et al., 2011). The only study that investigated selenium and mercury in relation to the incidence of ischemic stroke found no significant interaction (D. Mozaffarian et al., 2011).

Methylmercury exposure increases mercury levels in all tissues with a half-life of approximately 50 days (Sherlock et al., 1984). Thus, serum mercury can be considered an objective measurement of methylmercury and could reduce potential bias compared to estimated levels from dietary intake (Ahlqwist et al., 1999; Clarkson et al., 2007). Therefore, we examined the prospective association of serum mercury levels with the incidence of ischemic stroke and explored the possible effect modifications by serum selenium levels and demographic and geographic factors using a case-cohort study nested in the REasons for Geographic and Racial Differences in Stroke (REGARDS) project.

2. Methods

2.1. Study design and population

REGARDS is a national population-based prospective cohort study, which enrolled and is following a cohort of 30,239 black and white Americans aged ≥45 years. Individuals residing in the “Stroke Belt” region where the stroke mortality exceeds the rest of the U.S. (Alabama, Arkansas, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, and Tennessee) and blacks were oversampled. Participants were recruited between January 2003 and October 2007 using a combination of mail and telephone contact. After verbally consenting, participants were interviewed for information on demographic and risk factors through telephone contact. An in-person physical assessment was followed 3–4 weeks later, in which blood and urine samples as well as anthropometric measurements (blood pressure, height, weight, and electrocardiogram) were collected by standardized protocols. At the in-person physical assessment, written informed consents were obtained and self-administered questionnaires were left with participants to gather additional information on demographic, dietary and lifestyle characteristics. Participants (or their proxies) are contacted every 6 months for ascertainment of incident stroke events. Incident strokes and stroke types were verified by relevant hospital records and adjudicated centrally by neurologists. The detailed design and methods of the REGARDS study are provided elsewhere (Howard et al., 2005). This study was approved by institutional review boards of all participating institutions.

The present analysis uses a case-cohort study design. The case-cohort sample consists of a random sample from the parent REGARDS cohort, named the sub-cohort, and all of the incident ischemic stroke cases identified through September 2012. The sub-cohort (n=2,666) was randomly selected among participants with available data in the parent REGARDS cohort (n=29,653) with a fixed sampling probability of 9% in each stratum jointly classified by age (<55, 55–64, 65–74, 75–84, and ≥85, years), sex (male and female), race (black and white), and stroke region residency [Stroke Buckle (coastal plains of North Carolina, South Carolina, and Georgia), the rest of Stroke Belt, and non-Stroke-Belt region] (Cai and Zeng, 2004; Cushman et al., 2014). Through September 2012, 713 incident cases of ischemic stroke were identified. Among those, 63 cases were included in the random sub-cohort by

the random selection. Of the 3,316 participants in the case-cohort study, we excluded 219 whose mercury measurements were not available. Thus, the final dataset includes 662 cases of ischemic stroke and 2,494 participants in the random sub-cohort (including 59 incident cases; Figure 1).

2.2. Laboratory analyses

Urine and fasting blood samples were collected, shipped overnight with ice packs to a central laboratory, and stored at −80 °C for reprocessing and analysis (Kabagambe et al., 2011). Serum mercury was measured by using a Nippon MA-3000 direct mercury analyzer. Samples were analyzed in batches of 29–34 samples with instrument blanks, mercury check standards (CV=3.75%), NIST 1515 apple leaves (CV=2.2%), and in-house pooled QC serum (CV=6.93%).

The sample limit of detection was 0.0034 μg/dL. A total of 51 samples contained mercury at a level below the detection limit. Serum selenium was measured by instrumental neutron activation analysis with standard reference materials (NIST 1571 bovine liver, NIST 908b, and NIST 909c) (Morris et al., 2008). Several other elements, including urinary cadmium and arsenic, and serum calcium, magnesium, iron, and zinc concentrations were measured by the ICP-MS (Perkin Elmer, MA, USA). Urinary creatinine was quantified by using the Modular-P chemistry analyzer (Aaron et al., 2011). Lipid profiles were assessed by using colorimetric reflectance spectrophotometry (Cushman et al., 2009) and C-reactive protein by particle enhanced immunonephelometry (Cushman et al., 2009).

2.3. Outcome, exposure and covariates

The outcome was time to incident ischemic stroke obtained from self-report and adjudicated through hospital records by neurologists (Kasner et al., 2003). Survival time was defined as the period between the baseline interview and ischemic stroke event (first incidence), last follow-up, death, or data freeze (September 2012), whichever came first. The primary exposure was serum mercury concentration classified in quintiles or as a continuous variable excluding the values above 95th percentile in order to reduce the influence of extreme values.

Important covariates included age, sex, race (black or white), region (Stroke Buckle, the rest of Stroke Belt, or non-Stroke-Belt region), education levels (less than high school, high school, some college, or college graduate), BMI (<25.0, 25.0–29.9, or ≥30.0 kg/m^2), smoking status (never-, former, or current smoker), alcohol consumption (never-, former, or current drinker), physical activity (none, 1–3, or ≥4 times/week), hypertension (yes or no), history of myocardial infarction (yes or no), history of atrial fibrillation (yes or no), diabetes (yes or no), LCn3PUFA intake, HDL/LDL-cholesterol ratio, and C-reactive protein. The intake of LCn3PUFAs, defined as the sum of eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid, as well as fish consumption were estimated using the Block 98 Food Frequency Questionnaire (Howard et al., 2005). Demographics, education levels, lifestyle factors, and medical histories were *via* self-report. Hypertension was defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg or self-reported use of antihypertensive medication. Histories of myocardial infarction and atrial fibrillation were defined by electrocardiographic evidence or self-report. Diabetes was

defined as any self-reported use of glucose control medication or a fasting blood glucose concentration ≥126 mg/dL or non-fasting glucose ≥200 mg/dL. Weight and height were measured by trained professionals using standardized protocols and were used to calculate BMI (kg/m^2).

2.4. Statistical analyses

Baseline characteristics of the study population were summarized using mean values with standard deviations or medians with inter-quartile ranges for continuous variables and proportions for categorical variables. Analysis of variance, Kruskal-Wallis test, or chi-squared test,as appropriate, were used to compare participants' characteristics across quintiles of serum mercury levels in the random sub-cohort. Covariates significantly associated with serum mercury in the random sub-cohort were considered in the multivariable analysis. The Barlow weighting method for Cox proportional hazards regression models for case-cohort designs was used to examine the association between serum mercury levels and the incidence of ischemic stroke (Barlow, 1994). Since the potential competing risk of death is not negligible, we used a cause-specific hazard model. Continuous serum mercury concentration was used to test for linear trend with values above 95^{th} percentile deleted. Hazard ratios (HRs) and the corresponding 95% confidence intervals (CIs) using the lowest quintile of mercury as the referent were estimated.

Model 1 was adjusted for age, sex, race, age-race interaction and region. Model 2a was additionally adjusted for education level, smoking status, alcohol consumption, physical activity, hypertension, history of myocardial infarction, and diabetes. Model 2b (final model) was further adjusted for LCn3PUFA intake.

Several sensitivity analyses were conducted to test the robustness of our findings. To balance the covariates between comparison groups and alleviate the concern of collinearity, propensity score analysis, estimated by logistic regression including all covariates in the final model, was conducted to compare the highest quintile of serum mercury to the lowest. In addition, dietary fish consumption, instead of LCn3PUFA intake, was used for adjustment in the final model to reduce the confounding by other nutrients in fish. Moreover, concentrations of serum calcium, magnesium, selenium, zinc, and iron, and urinary arsenic and cadmium were additionally adjusted for in the final model to reduce confounding by other trace elements or heavy metals. Stratified analyses were performed using quintiles or continuous serum mercury concentration according to sex (female *vs.* male), race (black *vs.* white), region (Stroke Buckle *vs.* the rest of Stroke Belt *vs.* non-Stroke-Belt region), serum selenium levels (< median 13.1 μg/dL *vs.* ≥ median 13.1 μg/dL), and fish consumption (< median 7.0 g/day *vs.* ≥ median 7.0 g/day) with the adjustment for all covariates in the final model except the potential effect modifier. Interaction was tested using continuous mercury concentration with those values above 95^{th} percentile deleted.

The proportional hazards assumption was assessed by testing the coefficient of the interaction between mercury quintiles and survival time (Grambsch and Therneau, 1994). Main-effects *P* values ≤ 0.05 and interaction *P*values ≤ 0.10 were considered statistically significant. All analyses were conducted in SAS version 9.4 (SAS Institute, Cary, NC, USA).

3. Results

3.1. Baseline characteristics of participants

Baseline characteristics of stroke cases as well as the participants in the random sub-cohort are shown in Table 1. In the random sub-cohort, 55% of the participants were female and 40% were blacks with an average age of 65 years (standard deviation=9.4 years). The average follow-up time in the case-cohort was 6 years (standard deviation=2.4 years). The median serum mercury concentration was 0.03 μg/dL (inter-quartile range=0.02–0.06 μg/dL). Participants with higher serum mercury concentration were more likely to be younger, residents of non-Stroke-Belt regions, never- or former smokers, current alcohol drinkers, physically active, as well as to have higher education levels and higher levels of LCn3PUFA intake, fish consumption, and serum selenium. They were less likely to have history of myocardial infarction and diabetes.

3.2. Mercury exposure and incident ischemic stroke

The hazard ratios of incident ischemic stroke across quintiles of mercury concentration using the lowest quintile as the referent are shown in Table 2. After adjustment for potential confounders except LCn3PUFA intake, mercury had a moderate inverse association with the incidence of ischemic stroke (Model 2a; HR=0.70; 95% CI=0.51–0.97; quintile 5 *vs.* quintile 1; *P* for linear trend=0.04). Further adjustment for LCn3PUFA intake substantially attenuated the association (Model 2b; HR=0.82; 95% CI=0.55–1.22; quintile 5 vs. quintile 1; *P* for linear trend=0.42). The proportional hazards assumption was satisfied.

In sensitivity analyses, the observed association was not appreciably changed by using propensity score for adjustment (HR=0.84; 95% CI=0.55–1.29; quintile 5 *vs.* quintile 1; *P* for linear trend=0.42) or adjusting for fish consumption instead of LCn3PUFA intake (HR=0.86; 95% CI=0.57–1.29; quintile 5 *vs.* quintile 1; *P* for linear trend=0.52). In addition, the association was not confounded by other trace elements or heavy metals (HR=0.81; 95% CI=0.53–1.23; quintile 5 *vs.* quintile 1; *P* for linear trend=0.49).

Results of stratified analyses are shown in Table 3. The observed association was modified by sex (*P* for interaction=0.06). An inverse association between mercury and the incidence of ischemic stroke was observed in women (HR=0.50; 95% CI=0.27–0.93; quintile 5 *vs.* quintile 1; *P* for linear trend=0.04), but not in men (HR=1.20; 95% CI=0.67–2.13; quintile 5 *vs.* quintile 1; *P* for linear trend=0.42). No any other effect modifier was found, including race, region, serum selenium levels, and fish consumption.

4. Discussion

The present study does not support the hypothesis that mercury exposure is associated with the incidence of ischemic stroke within a population with low-to-moderate level of exposure (Ng et al., 2013). No increased risk of ischemic stroke in relation to mercury was observed in lower level of selenium, either. However, sex significantly modified the association; a more evident trend toward lower incidence of ischemic stroke with higher mercury levels was observed in women.

The mercury level in the present study is lower than that in a previous U.S. study (D. Mozaffarian et al., 2011), which is expected since the level of environmental mercury in the U.S. has declined since the 1980s (Wentz et al., 2014). Although biologically plausible, our findings are consistent with previous observational studies that failed to observe an increased risk of ischemic stroke with different mercury biomarkers (Daneshmand et al., 2016; D. Mozaffarian et al., 2011; Wennberg et al., 2007). Mercury was measured in serum in this study, but the mercury levels from different biomarkers should be correlated, permitting comparisons across studies (Clarkson et al., 2007). Similar to any other observational studies, the lack of association observed in the present study may be attributable to the residual confounding of LCn3PUFAs, even though the adjustment in the data analysis. Mercury was inversely associated with the incidence of ischemic stroke when dietary LCn3PUFA intake was not included in the analysis, but the association was no longer statistically significant after the adjustment, which supports this explanation. Also, other nutrients in fish such as vitamin D and some specific amino acids may exert influence on the association (Dariush Mozaffarian et al., 2011). Complete information on these nutrients was not available in this study and confounding is possible, though the sensitivity analysis adjusting for fish consumption and additionally adjusting for some other trace elements yielded essentially similar results. It is also possible that mercury may be an independent biomarker of fish consumption even after the adjustment for estimated intake. The observation that selenium is an antagonist of mercury toxicity is well established in animal studies (Ganther et al., 1972; Seppanen et al., 2000; Sumino et al., 1977). Differences in population selenium levels have been hypothesized to explain inconsistent findings of mercury exposure in relation to other cardiovascular outcomes (Virtanen et al., 2005). However, only one epidemiological study has examined whether selenium modified the association between mercury and the risk of ischemic stroke (D. Mozaffarian et al., 2011). Similar to the previous U.S. study, we did not find evidence of elevated incidence of ischemic stroke with higher mercury levels in participants with selenium levels below the median. In addition, serum selenium levels were associated with a modestly reduced incidence of ischemic stroke, although not statistically significant (data not shown). Of note, in prior European studies where selenium was hypothesized to modify the toxicity of mercury on cardiovascular endpoints, the selenium levels were lower than the average U.S. level. Thus, it is possible that the selenium level in the U.S. is sufficiently high to counteract the detrimental influence of mercury on ischemic stroke risk, which resulted in the null association in this study.

Sex-stratified analyses suggested an inverse association between mercury exposure and stroke in women, but not in men. A difference by sex has been seen previously, though the interaction was not statistically significant (D. Mozaffarian et al., 2011). The sex-related susceptibility to mercury toxicity has not been sufficiently studied in humans and the findings are inconclusive. Animal experiments suggested mitochondria from female rats exhibited higher expression of antioxidant enzymes and lower production of reactive oxygen species, which might protect against mercury-induced oxidative stress in females greater than males (Borrás et al., 2003). In addition, sex-dependent methylmercury metabolism has been reported in experimental animals (Thomas et al., 1987). Methylmercury-treated rats showed faster whole body clearance of mercury in females than in males, indicating sex

differences in excretion of organic mercury (Thomas et al., 1987). Besides the possible differences in mercury metabolism, women had a relatively lower level of mercury than men in this population and women generally have a more beneficial dietary habit and lifestyle, such as having a higher consumption of vegetables and fruits (Michels Blanck et al., 2008), having a higher intake of vitamins or other nutrient supplements (Bailey et al., 2013), preparing fish meals in a healthier way (Watterworth et al., 2017), and more likely to be never-smokers (Rostron et al., 2014). Thus, the influence of mercury on ischemic stroke risk might be less pronounced in women who had a lower mercury exposure and healthier lifestyle.

The present study reported results adjusted for dietary LCn3PUFA intake and fish consumption to reduce the confounding effect by nutrients in fish, while previous studies were often adjusted for fish intake alone due to lack of information on LCn3PUFA. This study also provides additional evidence regarding the effect modification by selenium, which is an important factor to consider when understanding the toxicity of mercury. In addition, objective measurements of mercury and covariates as well as adjudication of stroke substantially reduce the possibility of measurement errors and misclassification. Another strength of the present study is that it is based on a large sample of black and white men and women from across the continental United States, which facilitates the exploration of potential effect modifiers and improves generalizability. The main limitation of this study is the use of serum mercury for long-term exposure of methylmecury as well as the fact that it was measured only at baseline. Methylmercury exposure increases mercury level in all tissues including serum, but only about 10% of serum mercury is methylmercury (Clarkson et al., 2007). However, serum mercury level is correlated with fish consumption in this study. Meanwhile, occupational sources of exposure are unlikely to account for the findings because the imputed occupational exposure to non-specific contaminants were not associated with serum mercury in a sub-sample. Thus, the use of serum as an objective measurement of mecury from foods is supported in this study. Also, serum mercury reflects a relatively short time period compared to toenail or hair mercury (International Programme on Chemical Safety, 1990), but the mercury levels from different biomarkers should have a similar pattern (Clarkson et al., 2007). Since fish intake habits prevail for a long time, we should be able to reasonably assume that serum mercury levels did not change substantially during the study period. In addition, our findings might be partly confounded by the beneficial effects of LCn3PUFAs and other nutrients in fish, even after adjustment for estimated dietary intake. Similarly, the possibility of residual confounding from environmental, lifestyle, and dietary factors cannot be completely ruled out. But, the consistent results from the main and sensitivity analyses provide reassurance about the validity of our findings. Moreover, we were not able to examine the effect of mercury exposure on hemorrhagic stroke due to the small number of cases in the present study, though the associaiton does not appear to be substantially different across sub-types of stroke based on findings from previous studies (Daneshmand et al., 2016; Wennberg et al., 2007).

5. Conclusions

In conclusion, this study does not support an association between mercury and the incidence of ischemic stroke in a population with low-to-moderate levels of exposure. Selenium levels did not modify the association, presumably due to the relatively high level of selenium in this population. However, the present study cannot exclude the possibility of mercury-induced ischemic stroke toxicity at higher exposure levels in other populations. Environmental contamination of mercury should be continuously monitored and controlled since mercury still has the potential to counteract the cardiovascular benefits of fish consumption.

Acknowledgments

Funding

This work was supported by the National Institutes of Health [grant number R01ES021735, 2012]. The REGARDS research project is supported by a cooperative agreement U01 NS041588 from the National Institute of Neurological Disorders and Stroke, National Institutes of Health, and Department of Health and Human Service. Representatives of the funding agency have been involved in the review of the manuscript but not directly involved in the collection, management, analysis or interpretation of the data.

The authors thank the other investigators, the staff, and the participants of the REGARDS project for their valuable contributions. A full list of participating REGARDS investigators and institutions can be found at http://www.regardsstudy.org.

References

Aaron KJ, Campbell RC, Judd SE, Sanders PW, Muntner P. Association of dietary sodium and potassium intakes with albuminuria in normal-weight, overweight, and obese participants in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) Study. Am J Clin Nutr. 2011; 94(4):1071–1078. [PubMed: 21880845]

Ahlqwist M, Bengtsson C, Lapidus L, Gergdahl IA, Schutz A. Serum mercury concentration in relation to survival, symptoms, and diseases: results from the prospective population study of women in Gothenburg, Sweden. Acta Odontol Scand. 1999; 57(3):168–174. [PubMed: 10480284]

Bailey RL, Gahche JJ, Miller PE, Thomas PR, Dwyer JT. Why US adults use dietary supplements. JAMA Intern Med. 2013; 173(5):355–361. [PubMed: 23381623]

Barlow WE. Robust variance estimation for the case-cohort design. Biometrics. 1994; 50(4):1064–1072. [PubMed: 7786988]

Borrás C, Sastre J, García-Sala D, Lloret A, Pallardó FV, Viña J. Mitochondria from females exhibit higher antioxidant gene expression and lower oxidative damage than males. Free Radic Biol Med. 2003; 34(5):546–552. [PubMed: 12614843]

Cai J, Zeng D. Sample size/power calculation for case-cohort studies. Biometrics. 2004; 60(4):1015–1024. [PubMed: 15606422]

Clarkson TW, Vyas JB, Ballatori N. Mechanisms of mercury disposition in the body. Am J Ind Med. 2007; 50(10):757–764. [PubMed: 17477364]

Cushman M, Jenny NS, Zakai NA, Judd SE, Howard VJ, Gutiérrez OM, Ahmed A, Thacker EL, Kissela B. N-terminal pro-B-type natriuretic peptide and stroke risk: the Reasons for Geographic and Racial Differences in Stroke cohort. Stroke. 2014; 45(6):1646–1650. [PubMed: 24757103]

Cushman M, McClure LA, Howard VJ, Jenny NS, Lakoski SG, Howard G. Implications of increased C-reactive protein for cardiovascular risk stratification in black and white men and women in the US. Clin Chem. 2009; 55(9):1627–1636. [PubMed: 19643839]

Daneshmand R, Kurl S, Tuomainen TP, Virtanen JK. Associations of serum n-3 and n-6 PUFA and hair mercury with the risk of incident stroke in men: the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD). Br J Nutr. 2016; 115(10):1851–1859. [PubMed: 26991769]

Ganther HE, Goudie C, Sunde ML, Kopecky MJ, Wagner P. Selenium: relation to decreased toxicity of methylmercury added to diets containing tuna. Science. 1972; 175(4026):1122–1124. [PubMed: 5062150]

Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. Biometrika. 1994; 81(3):515–526.

Guallar E, Sanz-Gallardo MI, van't Veer P, Bode P, Aro A, Gomez-Aracena J, Kark JD, Riemersma RA, Martin-Moreno JM, Kok FJ. Mercury, fish oils, and the risk of myocardial infarction. N Engl J Med. 2002; 347(22):1747–1754. [PubMed: 12456850]

Hansen JC, Pedersen HS, Mulvad G. Fatty acids and antioxidants in the Inuit diet. Their role in ischemic heart disease (IHD) and possible interactions with other dietary factors. A review. Arctic Med Res. 1994; 53(1):4–17. [PubMed: 8049000]

Houston MC. Role of mercury toxicity in hypertension, cardiovascular disease, and stroke. J Clin Hypertens (Greenwich). 2011; 13(8):621–627. [PubMed: 21806773]

Howard VJ, Cushman M, Pulley L, Gomez CR, Go RC, Prineas RJ, Graham A, Moy CS, Howard G. The reasons for geographic and racial differences in stroke study: objectives and design. Neuroepidemiology. 2005; 25(3):135–143. [PubMed: 15990444]

Kabagambe EK, Judd SE, Howard VJ, Zakai NA, Jenny NS, Hsieh M, Warnock DG, Cushman M. Inflammation biomarkers and risk of all-cause mortality in the Reasons for Geographic And Racial Differences in Stroke cohort. Am J Epidemiol. 2011; 174(3):284–292. [PubMed: 21685411]

Kasner SE, Cucchiara BL, McGarvey ML, Luciano JM, Liebeskind DS, Chalela JA. Modified National Institutes of Health Stroke Scale can be estimated from medical records. Stroke. 2003; 34(2):568–570. [PubMed: 12574577]

Lackland DT, Roccella EJ, Deutsch AF, Fornage M, George MG, Howard G, Kissela BM, Kittner SJ, Lichtman JH, Lisabeth LD, Schwamm LH, Smith EE, Towfighi A. Factors influencing the decline in stroke mortality: a statement from the American Heart Association/American Stroke Association. Stroke. 2014; 45(1):315–353. [PubMed: 24309587]

Lanska DJ. Geographic distribution of stroke mortality in the United States: 1939–1941 to 1979–1981. Neurology. 1993; 43(9):1839–1851. [PubMed: 8414045]

Michels Blanck H, Gillespie C, Kimmons JE, Seymour JD, Serdula MK. Trends in Fruit and Vegetable Consumption Among US Men and Women, 1994–2005. Prev Chronic Dis. 2008; 5(2):A35. [PubMed: 18341771]

Morris JS, Ngwenyama RA, Guthrie JM, Brockman JD, Spate VL, Robertson JD. Quality control in the neutron activation analysis of biological markers for selenium in epidemiological investigations. Journal of Radioanalytical & Nuclear Chemistry. 2008; 276(1):7–13.

Mozaffarian D, Appel LJ, Van Horn L. Components of a cardioprotective diet. Circulation. 2011; 123(24):2870–2891. [PubMed: 21690503]

Mozaffarian D, Shi P, Morris JS, Spiegelman D, Grandjean P, Siscovick DS, Willett WC, Rimm EB. Mercury exposure and risk of cardiovascular disease in two U.S. cohorts N Engl J Med. 2011; 364(12):1116–1125. [PubMed: 21428767]

Ng TH, Mossey JM, Lee BK. Total blood mercury levels and depression among adults in the United States: National Health and Nutrition Examination Survey 2005–2008. PLoS One. 2013; 8(11):e79339. [PubMed: 24244482]

Rostron BL, Chang CM, Pechacek TF. Estimation of cigarette smoking-attributable morbidity in the United States. JAMA Intern Med. 2014; 174(12):1922–1928. [PubMed: 25317719]

International Programme on Chemical Safety. Methylmercury. World Health Organization; Switzerland: 1990.

Seppanen K, Kantola M, Laatikainen R, Nyyssonen K, Valkonen VP, Kaarlopp V, Salonen JT. Effect of supplementation with organic selenium on mercury status as measured by mercury in pubic hair. J Trace Elem Med Biol. 2000; 14(2):84–87. [PubMed: 10941718]

Sherlock J, Hislop J, Newton D, Topping G, Whittle K. Elevation of mercury in human blood from controlled chronic ingestion of methylmercury in fish. Hum Toxicol. 1984; 3(2):117–131. [PubMed: 6724592]

Solenkova NV, Newman JD, Berger JS, Thurston G, Hochman JS, Lamas GA. Metal pollutants and cardiovascular disease: mechanisms and consequences of exposure. Am Heart J. 2014; 168(6): 812–822. [PubMed: 25458643]

National Center for Health Statistics. [accessed 10 June 2017] Mortality multiple cause micro-data files 2013. 2011. http://www.cdc.gov/nchs/data_access/Vitalstatsonline.htm#Mortality_Multiple

Sumino K, Yamamoto R, Kitamura S. A role of selenium against methylmercury toxicity. Nature. 1977; 268(5615):73–74. [PubMed: 18676]

Thomas DJ, Fisher HL, Sumler MR, Mushak P, Hall LL. Sexual differences in the excretion of organic and inorganic mercury by methyl mercury-treated rats. Environ Res. 1987; 43(1):203–216. [PubMed: 3582308]

Virtanen JK, Voutilainen S, Rissanen TH, Mursu J, Tuomainen TP, Korhonen MJ, Valkonen VP, Seppanen K, Laukkanen JA, Salonen JT. Mercury, fish oils, and risk of acute coronary events and cardiovascular disease, coronary heart disease, and all-cause mortality in men in eastern Finland. Arterioscler Thromb Vasc Biol. 2005; 25(1):228–233. [PubMed: 15539625]

Watterworth JC, Hutchinson JM, Buchholz AC, Darlington G, Randall Simpson JA, Ma DWL, Haines J. Food parenting practices and their association with child nutrition risk status: comparing mothers and fathers. Appl Physiol Nutr Metab. 2017; 42(6):667–671. [PubMed: 28196327]

Wennberg M, Bergdahl IA, Stegmayr B, Hallmans G, Lundh T, Skerfving S, Stromberg U, Vessby B, Jansson JH. Fish intake, mercury, long-chain n-3 polyunsaturated fatty acids and risk of stroke in northern Sweden. Br J Nutr. 2007; 98(5):1038–1045. [PubMed: 17537290]

Wentz DA, Brigham ME, Chasar LC, Lutz MA, Krabbenhoft DP. Mercury in the Nation's streams—Levels, trends, and implications. US Geological Survey Circular. 2014; 1395:90.

Highlights

- Serum mercury is not associated with an increased incidence of ischemic stroke.
- Serum selenium level did not modify the association.
- A more evident trend of inverse association was observed in women, but not in men.

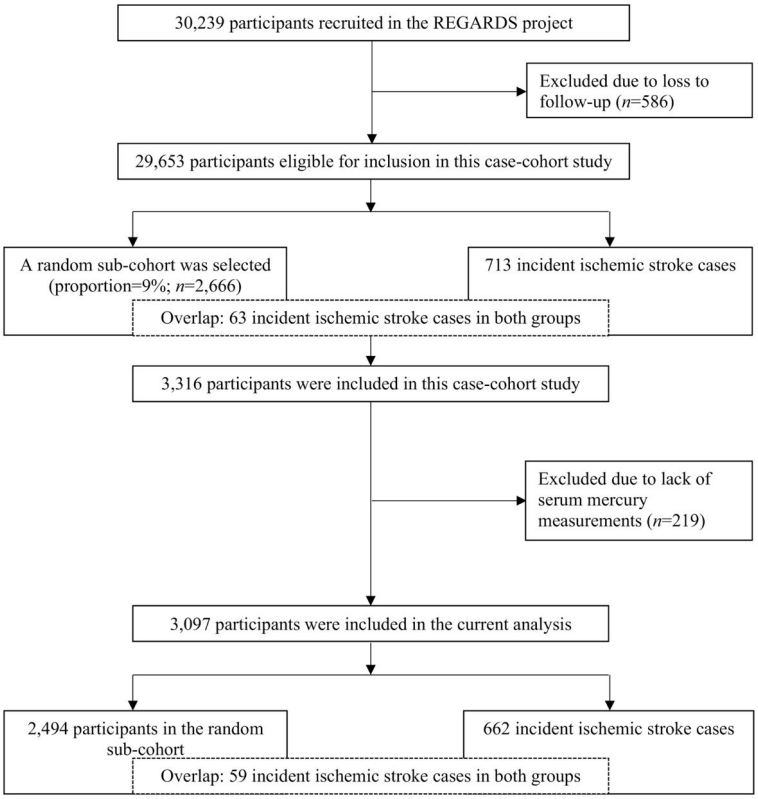

Figure 1.
Flow chart of sub-cohort and cases sampling.

Environ Int. Author manuscript; available in PMC 2019 August 01.

Table 1

Baseline characteristics of the study population by quintiles of serum mercury levels, the REGARDS Trace Element Study.[a]

	Cases	Random Sub-cohort	Quintiles of serum mercury levels (μg/dL)[b]					P value[c]
			Q1 (≤0.02)	Q2 (0.02–0.03)	Q3 (0.03–0.04)	Q4 (0.04–0.07)	Q5 (>0.07)	
n	662	2,494	501	497	502	496	498	--
Median mercury level (μg/dL)	0.03	0.03	0.01	0.02	0.03	0.05	0.10	--
Age (year)	70.1±8.6	64.9±9.4	66.1±10.0	65.9±9.5	64.7±9.4	64.6±8.9	63.1±8.7	<0.05
Female (%)	48.5	54.5	57.1	56.7	55.8	50.6	52.2	0.15
Black (%)	40.9	40.3	38.5	43.1	40.2	38.9	40.8	0.61
Region (%)								
Stroke Buckle	21.8	21.0	18.8	20.9	24.5	21.6	19.3	<0.05
The rest of Stroke Belt	34.3	34.7	45.9	38.8	32.5	29.8	26.5	
Non-Stroke-Belt region	44.0	44.3	35.3	40.2	43.0	48.6	54.2	
Education (%)								
Less than high school	15.1	12.3	22.7	14.1	9.0	7.7	8.0	<0.05
High school graduate	29.6	24.1	28.3	27.6	20.9	24.4	19.5	
Some college	26.0	27.5	26.9	29.2	32.1	26.0	23.3	
College graduate	29.3	36.1	22.2	29.2	38.1	41.9	49.2	
Smoking status (%)								
Never-	40.9	45.9	41.7	46.1	46.8	47.4	47.4	<0.05
Former	42.0	39.1	35.5	36.4	41.0	41.1	41.6	
Current	17.1	15.0	22.8	17.5	12.2	11.5	11.0	
Alcohol consumption (%)								
Never-	31.6	30.2	38.9	35.0	26.9	29.0	20.9	<0.05
Former	22.2	18.4	23.8	21.5	18.1	14.1	14.3	
Current	46.2	51.5	37.3	43.5	55.0	56.9	64.9	
Physical activity (%)								
None	34.8	33.6	40.9	33.5	33.7	32.6	27.1	<0.05
1–3 times/week	35.4	35.4	30.2	32.5	38.0	38.3	38.2	
≥4 times/week	29.8	31.0	29.0	34.0	28.3	29.1	34.8	
Hypertension (%)	73.3	58.5	62.0	63.8	52.0	55.7	59.2	<0.05

	Cases	Random Sub-cohort	Quintiles of serum mercury levels (μg/dL)[b]					P value[c]
			Q1 (≤0.02)	Q2 (0.02–0.03)	Q3 (0.03–0.04)	Q4 (0.04–0.07)	Q5 (>0.07)	
Myocardial infarction (%)	21.4	12.0	15.7	13.0	11.4	10.7	9.2	<0.05
Diabetes (%)	28.7	21.3	27.1	25.3	19.7	19.5	15.1	<0.05
LCn3PUFA intake (g/day)	0.1 (0.0–0.1)	0.1 (0.0–0.2)	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.1 (0.0–0.2)	0.1 (0.0–0.2)	0.1 (0.0–0.2)	<0.05
Fish consumption (g/day)	6.4 (2.9–13.5)	7.0 (3.0–15.4)	3.5 (1.5–9.8)	6.4 (2.9–14.0)	7.0 (3.0–15.4)	8.4 (3.7–15.6)	12.4 (5.8–26.0)	<0.05
Serum selenium (μg/dL)	13.0 (11.9–14.2)	13.1 (11.9–14.3)	12.7 (11.5–14.1)	13.2 (12.1–14.3)	13.1 (11.9–14.2)	13.2 (12.0–14.3)	13.3 (12.3–14.5)	<0.05

Abbreviations: LCn3PUFA, long chain n-3 polyunsaturated fatty acids; Q1–5, quintile 1–5; REGARDS, REasons for Geographic and Racial Differences in Stroke.

[a] Results are presented by means ± standard deviations, medians (inter-quartile ranges) or proportions.

[b] Quintiles of serum mercury were calculated based on the random sub-cohort (n=2,494).

[c] P values are for any difference across the quintiles of serum mercury levels in the random sub-cohort by using analysis of variance, Kruskal-Wallis test, or chi-squared test as appropriate.

Table 2

Multivariable-adjusted HRs (95% CIs) of incident ischemic stroke by quintiles of serum mercury levels, the REGARDS Trace Element Study.[a]

	Quintiles of serum mercury levels[b]					
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	***P* for linear trend**
Range (μg/dL)	≤0.02	0.02–0.03	0.03–0.04	0.04–0.07	>0.07	--
Median (μg/dL)	0.01	0.02	0.03	0.05	0.10	--
Sub-cohort	501	497	502	496	498	--
No. of cases	180	147	118	118	99	--
Model 1[c]	1.00 (Ref.)	0.80 (0.61–1.05)	0.69 (0.52–0.92)	0.67 (0.51–0.90)	0.60 (0.44–0.81)	0.001
Model 2a[d]	1.00 (Ref.)	0.89 (0.67–1.18)	0.76 (0.56–1.04)	0.79 (0.58–1.06)	0.70 (0.51–0.97)	0.04
Model 2b (final model)[e]	1.00 (Ref.)	1.01 (0.71–1.44)	0.78 (0.53–1.15)	0.95 (0.66–1.36)	0.82 (0.55–1.22)	0.42

Abbreviations: CI, confidence interval; HR, hazard ratio; No., number; Q1–5, quintile 1–5; Ref., referent; REGARDS, REasons for Geographic and Racial Differences in Stroke.

[a] All models were constructed using Barlow-weighted Cox proportional hazards regression analysis for case-cohort study. Linear association was examined by using continuous mercury concentration with those values above its 95th percentile deleted.

[b] Quintiles of serum mercury were calculated based on the random sub-cohort (n=2,494).

[c] Model 1 was adjusted for age, sex, race (black or white), age-race interaction and region (Stroke Buckle, the rest of Stroke Belt, or non-Stroke-Belt region).

[d] Model 2a was additionally adjusted for education levels (less than high school, high school, some college, or college graduate), smoking status (never-, former, or current smoker), alcohol consumption (never-, former, or current drinker), physical activity (none, 1–3 times/week, or ≥4 times/week), hypertension (yes or no), history of myocardial infarction (yes or no), and diabetes (yes or no).

[e] Model 2b was additionally adjusted for long chain n-3 polyunsaturated fatty acids intake.

Table 3

Associations [HRs (95% CIs)] between serum mercury levels and the incident ischemic stroke stratified by pre-specified factors, the REGARDS Trace Element Study.[a]

	Serum mercury levels [mean (SD), μg/dL]	No. of cases	No. of participants in sub-cohort	Quintiles of serum mercury levels[b]					*P* for linear trend
				Q1 (<0.02)	Q2 (0.02–0.03)	Q3 (0.03–0.04)	Q4 (0.04–0.07)	Q5 (>0.07)	
Sex									
Female	0.04 (0.07)	321	1,359	1 (Ref.)	0.92 (0.57–1.51)	0.84 (0.49–1.45)	0.79 (0.46–1.36)	0.50 (0.27–0.93)	0.04
Male	0.05 (0.05)	341	1,135	1 (Ref.)	1.14 (0.67–1.95)	0.72 (0.39–1.31)	1.14 (0.68–1.92)	1.20 (0.67–2.13)	0.42
P for interaction	--	--	--				0.06		
Race									
Black	0.04 (0.05)	271	1,005	1 (Ref.)	0.99 (0.53–1.87)	0.91 (0.46–1.82)	1.09 (0.55–2.14)	0.82 (0.41–1.66)	0.54
White	0.05 (0.07)	391	1,489	1 (Ref.)	1.02 (0.66–1.58)	0.73 (0.45–1.20)	0.88 (0.57–1.37)	0.81 (0.49–1.36)	0.55
P for interaction	--	--	--				0.48		
Region									
Stroke Buckle	0.04 (0.05)	144	524	1 (Ref.)	2.12 (0.80–5.63)	0.39 (0.12–1.31)	1.07 (0.36–3.15)	0.94 (0.31–2.90)	0.47
The rest of Stroke Belt	0.04 (0.04)	227	866	1 (Ref.)	0.76 (0.42–1.37)	1.01 (0.53–1.92)	0.84 (0.43–1.62)	1.01 (0.48–2.10)	0.71
Non-Stroke-Belt region	0.05 (0.08)	291	1,104	1 (Ref.)	1.08 (0.59–1.94)	0.92 (0.49–1.71)	1.11 (0.61–2.02)	0.78 (0.42–1.46)	0.41
P for interaction	--	--	--				0.62		
Serum selenium levels[b]									
<13.1 μg/dL (median)	0.04 (0.05)	345	1,207	1 (Ref.)	0.90 (0.54–1.50)	0.86 (0.48–1.52)	1.10 (0.64–1.88)	0.82 (0.46–1.47)	0.83
≥13.1 μg/dL (median)	0.05 (0.07)	317	1,287	1 (Ref.)	1.22 (0.72–2.06)	0.70 (0.39–1.25)	0.96 (0.56–1.64)	0.89 (0.49–1.61)	0.24
P for interaction	--	--	--				0.22		
Fish consumption[b]									
<7.0 g/day (median)	0.04 (0.08)	238	880	1 (Ref.)	1.35 (0.85–2.14)	0.67 (0.39–1.15)	0.74 (0.44–1.26)	0.81 (0.44–1.51)	0.42
≥7.0 g/day (median)	0.06 (0.05)	220	943	1 (Ref.)	0.74 (0.40–1.38)	0.83 (0.44–1.57)	1.13 (0.63–2.01)	0.83 (0.46–1.51)	0.66
P for interaction	--	--	--				0.68		

Abbreviations: CI, confidence interval; HR, hazard ratio; No., number; Q1–5, quintile 1–5; Ref., referent; REGARDS, REasons for Geographic and Racial Differences in Stroke; SD, standard deviation.

[a]All models were constructed using Barlow-weighted Cox proportional hazards regression analysis for case-cohort study with adjustment for the variables in model 2b, Table 2, except the potential modifier. Linear association and interaction were examined by using continuous mercury concentration with those values above its 95th percentile deleted.

[b]Quintiles of serum mercury , serum selenium and fish consumption levels were calculated based on the random sub-cohort (n=2,494).