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Mercury in serum predicts low risk of death and myocardial infarction in Gothenburg women

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Abstract

Purpose Markers of mercury (Hg) exposure have shown both positive and negative associations to cardiovascular disease (CVD). We assessed the association between serum Hg (S-Hg) and risk of cardiovascular disease in a prospective population-based cohort, with attention to the roles of dental health and fish consumption.

Methods Total mortality, as well as morbidity and mortality from acute myocardial infarction (AMI) and stroke, was followed-up for 32 years in 1,391 women (initially age 38-60), in relation to S-Hg at baseline, using Cox regression models. Potential confounders (age, socioeconomic status, serum lipids, alcohol consumption, dental health, smoking, hypertension, waist-hip ratio and diabetes) and other covariates (e.g. fish consumption) were also considered.

Results Hazard ratios (HR) adjusted only for age showed strong inverse associations between baseline S-Hg and total mortality [highest quartile: Hazard Ratio (HR) 0.76; 95% confidence interval (CI) 0.59-0.97], incident AMI (HR 0.56; CI 0.34-0.93) and fatal AMI (HR 0.31; CI 0.15-0.66). Adjustment for potential confounding factors, especially dental health, had a strong impact on the risk estimates, and after adjustment only the reduced risk of fatal AMI remained statistically significant.

Conclusions There was a strong inverse association between Hg exposure and CVD. Likely reasons are confounding with good dental health (also correlated with the number of amalgam fillings in these age-groups) and/or fish consumption. The results suggest potential effects of dental health and/or fish consumption on CVD that deserve attention in preventive medicine.

Keywords Mercury; Fish; Amalgam; Mortality; Cardiovascular; Myocardial infarction; Stroke

Introduction

Associations between the levels of mercury (Hg) in hair or nails and increased cardiovascular risk have been observed in epidemiological studies (Salonen et al. 1995; Guallar et al. 2002; Virtanen et al. 2005). One study also suggested possible cardiovascular risk from occupational exposure to inorganic Hg (Barregard et al. 1990). On the other hand, in a recent study among U.S. health professionals, no association was found between Hg levels in nails and risk of coronary heart disease, stroke or cardiovascular disease (Mozaffarian et al. 2011). Furthermore, in prospective case-control studies from Northern Sweden 'protective' associations have been found between levels of Hg in blood cells and risk of acute myocardial infarction (AMI; Hallgren et al. 2001; Wennberg et al. 2011), but not stroke (Wennberg et al. 2007).Similarly, the Population Study of Women in Gothenburg, in the southwest of Sweden, indicated protective associations between levels of Hg in serum (S-Hg), on the one hand, and total mortality and fatal AMI, on the other, in a 24-year follow-up of a cohort of 1462 women followed since 1968-69 (Ahlqwist et al. 1999).

Fish consumption has also been associated with decreased risk of cardiovascular outcomes in many, though far from all, studies (He 2009). It is believed that nutrients in fish, especially long-chain n-3 polyunsaturated fatty acids (PUFAs), cause the beneficial effects. However, fish is also the main source of methylmercury (MeHg), a contaminant known to induce oxidative stress (Virtanen et al. 2007). Therefore, in theory, MeHg could counteract the beneficial constituents in fish (Mozaffarian 2009; Roman et al. 2011) while Hg is also a marker of fish consumption.

The aim of this study is to describe the relation between S-Hg and mortality, AMI, or stroke in the 32-years follow-up of the Population Study of Women in Gothenburg, with special attention to possible roles of dental status and fish intake.

Methods

Overall design

This is a cohort study with baseline characteristics obtained in first examination in 1968-69. Serum samples were obtained at the same time but Hg determination in freeze-stored samples taken in 1968-69 was made in 1991-92. Mortality, stroke and AMI was followed until 32 years after baseline, i.e. 2000-2001.

Sampling

In 1968-69, a prospective population study of female residents in the city of Gothenburg, western Sweden was initiated. At that time, a representative sample was identified, consisting of 1,622 subjects within the age strata 38, 46, 50, 54 and 60 years. After being invited to attend a study involving women's health, 1,462 (90%) chose to participate in the survey, which consisted of a physical examination, blood and urine sampling, and detailed interviews describing medical history and lifestyle (Bengtsson et al. 1973). Four follow-up examinations have subsequently been carried out in 1974-75, 1980-81, 1992-93 and 2000-01 (Lissner et al. 2003). After 32 years 929/1462 of the original participants were still alive and less than 1% had been lost to follow-up. All participants gave their informed consent prior to their inclusion in the study. The study has been approved by the Ethics Committee of the University of Gothenburg (Ö402-99 and T227-03).

Medical and dental examination

Blood pressure was measured in the sitting position after 5 minutes of rest. Hypertension was defined as using antihypertensive medication, and/or a systolic blood pressure >160 mmHg and/or a diastolic blood pressure >95 mmHg at the time of the examination. Waist and hip circumferences were measured and the waist-hip ratio calculated (Bengtsson et al. 1973). A subject was defined as having diabetes if on anti-diabetic medication, or if the diagnosis was set by a physician, or by two succeeding fasting samples with plasma glucose ≥7.0 mmol/L (in 2000-01 examination). Diabetes was also accepted as diagnosis if recorded in the death certificate (Björkelund et al. 2005).

The baseline examination also included a dental survey, which 97% of the participants included in the main survey attended. The dental examination included a panoramic radiographic survey, a questionnaire, and a colored photograph of the teeth. Based on this information, number of missing teeth (out of 32), and number of amalgam fillings, could be calculated (Ahlqwist et al. 1988a and 1988b).

Socioeconomic and lifestyle indicators

Subjects reported if they were current, ex- or never-smokers. Education was treated as a dichotomous variable that consisted of 2 categories: basic education (in 1968-69 6 years) and more than basic education. The majority of women had only basic education, while 30% had gone beyond this level including less than 2% who had attended university or college. Socioeconomic status as defined by occupational group was based primarily on husband's occupation among the 1,156 women who were married, but a woman's own occupation was used if she was unmarried. This variable consisted of three levels: higher (large scale employers and officials of high or intermediate rank), medium (small scale employers,

officials of lower rank, foremen), and lower (skilled and unskilled workers) (Cabrera et al. 2001).

The previous day's fish intake was reported by each woman in a 24-hour recall and the responses were coded as grams of fish. The 24-hour recall covered the entire diet and was performed through an interview by a dietician (Lenner at el., 1977).

Laboratory procedures

The baseline health examination included fasting blood sampling and urine collection, aimed both for immediate and future analyses. Approximately 120 mL of blood was drawn from the cubital vein into two open glass tubes without coagulant (Bengtsson et al. 1973). A small part of the blood was used for initial analyses, and the remaining portion was allowed to clot at room temperature for 2-3 hours. After centrifugation, the serum was stored at -20 °C. Previous work has shown that the original samples were minimally evaporated and suitable for other analyses (Rybo et al. 1985). Analysis of total cholesterol and triglycerides were done at the time of the baseline examination (Bengtsson et al. 1973).

Mercury determinations

Hg was determined in serum samples in duplicate by cold vapor atomic absorption spectrometry (Bergdahl et al. 1995 and 1998). The detection limit was 0.5 nmol/L. Accuracy was ascertained by including reference samples in each run (Seronorm® Trace Elements Whole Blood, Batch No. 904, Nycomed, Oslo, Norway). The mean and standard deviation for our analyses were 17.0 ± 0.8 nmol/L (n=77), while the recommended (but not certified), value was 20 nmol/L. The precision of the duplicate analyses was good: more than 90% differed <0.5 nmol/L.

Follow-up and endpoints

32-year mortality, total and cause-specific, was ascertained with the assistance of the Swedish Death Registry and the Swedish State Population and Address Registry. Occurrence of AMI (total, fatal and non-fatal) and stroke were monitored by means of hospital and primary care records, death certificates, interviews with participants during follow-up examinations, and National Patient Registry (inpatient data 1987-2001). Risk time for AMI and stroke was calculated to the day when the woman was admitted to hospital for AMI or stroke. All self-reported diagnoses concerning AMI and stroke were verified through the above mentioned sources.

Statistics

First, we investigated Spearman´s rank correlations between exposure variables (S-Hg, fish consumption and dental amalgam), covariates (potential confounders, effect modifiers, and others), and outcomes (mortality, AMI, and stroke). The following factors were significantly ($P<0.05$) associated with both exposure and outcome: age, number of teeth, number of amalgam fillings, social class, education, serum triglycerides, and wine consumption. Since there was a close association ($r_s = 0.84$) between number of teeth and number of amalgam fillings, we only included number of teeth in the statistical model. In addition, smoking, waist-hip ratio, serum cholesterol, hypertension and diabetes were predictors of outcomes, but not related to exposure. Therefore, they were not potential confounders, and were not included in the full multivariate model.

The associations between S-Hg and outcomes were assessed by survival analysis (Cox regression). Hazard ratios (HR) and 95 % confidence intervals (CI) were calculated using

SAS 9.1 (SAS institute, Inc., Cary, NC, USA). HRs were first calculated adjusting only for age, and then in models including the potential confounders.

Results

The median S-Hg was 1.4 μg/L (6.9 nmol/L; **Table 1**). The women had a median of 13 (range: 0-65; IQR: 1-25) amalgam surfaces and 188 women had lost all their teeth. In the dietary interview, 24 % reported fish intake during the preceding 24 hours (median in those; 100 g/day).

S-Hg was statistically significantly associated with both fish intake (r_s=0.13) and number of amalgam fillings (r_s=0.32**,** Table 1), while fish intake and amalgam fillings were uncorrelated to each other (r_s=0.00). The strongest correlations with S-Hg (r_s=0.17-0.41) were found for number of remaining teeth, number of amalgam fillings, being a wine-drinker, education, and social class. In addition, serum triglyceride level was negatively associated with S-Hg (r_s=-0.14).

When adjusting for age only, statistically significant "protective" associations with S-Hg were found for total mortality (age-adjusted HR 0.76 in highest S-Hg quartile), all AMI (HR 0.56, 95% CI 0.34-0.93), especially fatal AMI (HR 0.31, 95% CI 0.15-0.66), but not non-fatal AMI or stroke (**Table 2**).

Most associations were attenuated after taking into account potential confounders, mainly of socioeconomic nature (**Table 2**). However, for fatal AMI, the reduction in risk remained statistically significant even after adjustment (HR 0.43 in highest quartile) . In addition, an increased risk of stroke with increasing S-Hg became statistically significant (HR 1.80).

There were no statistically significant associations between fish consumption and any of the outcomes (not in table). Nevertheless, in supplementary analyses we added fish consumption to the adjusted model (not shown). The effects on HRs were marginal, but CIs were widened and the associations between S-Hg and fatal AMI, or stroke were no longer statistically significant. This was also the case if we added the number of amalgam fillings to the adjusted model.

Discussion

This prospective investigation of mortality, AMI and stroke in a cohort of women with previously determined S-Hg suggested that a higher S-Hg predicted low mortality, and very low risks of fatal AMI, but had no predictive value for non-fatal AMI or stroke. However, when potential confounders were included in multivariate models, only the low risk of fatal AMI in the highest S-Hg quartile remained statistically significant, while an increased stroke risk was suggested. Inclusion of amalgam fillings or fish intake in the models did not change these patterns but widened the 95% CIs to overlap 1. Although the follow-up time was very extended, there was an almost complete follow-up of the cohort. Therefore it is unlikely that these results are biased by loss to follow up.

The choice of serum for Hg determination was based on availability of samples. The usual medium for biomonitoring of MeHg is hair, blood or erythrocytes, sometimes nails. Still, S-Hg appears to provide information on MeHg exposure, since it has been shown to be associated with fish intake in a subset of the same cohort at a later sampling (Bergdahl et al. 1998). Hg in plasma/serum is also a reasonably good index of exposure to inorganic Hg from

amalgam fillings (Åkesson et al. 1991; Bergdahl et al. 1998). Therefore, S-Hg here used is a marker of both fish consumption and amalgam fillings.

There was no indication of Hg contamination of the sampling tubes. Also, the determinations of S-Hg were very accurate (Bergdahl et al. 1995), though the lack of certified reference materials with adequate concentrations of Hg is a problem (no such samples were available for fresh or frozen serum at the time of the Hg determinations).

The S-Hgs in 1968-69 are in accordance with those recorded in other relevant studies of Swedes (Skerfving 1974; Barregard et al. 1988). However, a single determination of a biomarker with fairly rapid turnover, in the beginning of a long follow-up period, certainly implies potential misclassification as to long term exposure. Also, there has been a decrease of MeHg exposure in Sweden over time (Bergdahl et al. 1998; Wennberg et al 2006). Therefore, the initial S-Hgs here are not representative of the whole follow-up period.

The determinations of amalgam fillings (which was historically, and at the time of the baseline, the dominating type of dental restoration in Sweden) and number of teeth were very accurate, but we have limited information on an individual's usual fish consumption: In contrast to a diet history or food frequency method, the 24-hour recall used here only capture intake on a single day before the baseline investigation and gives no information on an individual's cumulative exposure to fish. Dietary underreporting further adds to the error associated with this method. (Lenner et al., 1977). However, it may be noted that the proportion of women reporting fish consumption on the day of their recall, together with the amount reportedly consumed, is in accordance with the average in Swedish women a couple of decades ago (NFA 1994). However, a single 24-hour recall cannot accurately describe an

individual's usual intake of foods (like fish) with high day-to-day variability, which is a likely explanation for weaker associations between S-Hg and fish intake, compared to S-Hg and number of amalgam fillings. Despite these shortcomings in the exposure assessment, a number of associations appeared between health outcomes and S-Hg.

These associations needed to be investigated in relation to confounders. There is a series of potential confounders that are associated with both exposure (S-Hg, fish intake and amalgam fillings) and outcomes. The strongest one is the number of teeth, which is in accordance with earlier findings in the same cohort (Cabrera et al. 2005). Number of teeth is an indicator of both dental health and socioeconomic status, and it is also associated with both amalgam fillings and fish consumption. Hence, if fish consumption is protective, there is an obvious risk of over-adjusting when including socioeconomic factors in the models, which in our analyses resulted in attenuation of the S-Hg associated risk of AMI. Also, the inclusion of serum triglycerides in the model may be disputed, since it may be a step on the causal chain between fish intake (as reflected by S-Hg) and outcome, i.e. over-adjustment. Hence, the "true" effect could be somewhere in between the crude (age-adjusted only) and the fully adjusted models. We only adjusted for factors that could be confounders, i.e. were related to both outcome and exposure. Smoking was associated with AMI (P=0.10), but not with S-Hg (P=0.97), and was therefore not included in the statistical model. Similarly, waist-hip ratio, serum cholesterol, hypertension and diabetes were predictors of outcomes, but not related to exposure and were not included in the model (see Statistics section).

Certainly, MeHg is not protective against AMI; in contrast, as noted above, there are indications that Hg causes cardiovascular disease (Salonen et al. 1995; Guallar et al. 2002, Virtanen et al. 2005). Rather, the present seemingly preventive effect of S-Hg on AMI risk is likely to be caused by better dental health (and therefore more amalgam fillings) and/or intake

of fish. Earlier, we found that as much as half of the Hg in serum was present as MeHg (Bergdahl et al. 1998), which is the Hg species present in fish (WHO 1990). Nutrients in fish, such as PUFAs, are probable causes of the positive health effect (He 2009; Mozaffarian 2009). The net effect of the fish intake will depend on the relative intake of hazardous and health promoting fish components. Since the relative contents of these vary between different fish species, the effect will depend on the fish consumption habits of the population and individual. In Western (present study) and Northern (Hallgren et al. 2001; Wennberg et al. 2007, 2011) Sweden, it seems that the net effect is positive (in spite of the MeHg exposure), while in Eastern Finland the net effect appears to be negative (Virtanen et al. 2005).
It is noteworthy that the present data indicate that the effect is mainly on fatal AMI. This is in accordance with the observation that PUFAs counteract arrhythmias (Albert et al. 2002), which is the main cause of death in AMI patients.

The present results are compatible with earlier reports of associations between Hg exposure and AMI. Hence, on the basis of other data in the same cohort (Bergdahl et al. 1998), the present average S-Hg levels correspond to an erythrocyte Hg level of about 6 µg/L, which is in the upper range of other recent studies in Sweden (Hallgren et al. 2001; Wennberg et al. 2011), in which there were remarkable decreases of AMI risk with increasing MeHg exposure. For stroke, however, an earlier study in Swedes showed no association to Hg (Wennberg et al. 2007). It may be that the presently observed association (only in the adjusted model) is dependent on the long follow-up, while both studies have very accurate recording of cases (excluding loss to follow up as an explanation of the difference between study results).

The lack of consistency between the different CVD outcomes (stroke, fatal AMI and non-fatal AMI) may be viewed as a sign of lack of robustness of the results. Alternatively, this reflects

differences between these diseases in their etiology and mechanisms of Hg-related risk factors.

The present S-Hg concentrations correspond to exposure in the lower end compared to Hg concentrations in hair (WHO 1990) and nails (Choi et al. 2009), earlier shown to be associated with an increase of AMI in some studies (hair; Virtanen et al. 2005; nails: Guallar et al. 2002), but not in a recent large U.S. study (Mozaffarian et al. 2011). As compared to the occupational inorganic Hg exposure in the chloralkali industry, for which a slight increase of cardiovascular mortality was observed (Barregard et al. 1990), the present study was made at much lower exposures: the present median S-Hg was 1.4 μg/L, while the concentrations in blood of Swedish chloralkali workers from the 1970s on would be around 8 μg/L (40 nmol/L, geometric mean; Sallsten et al. 1990), roughly corresponding to the same concentration in serum, and before the 1970s even higher.

In conclusion, this study adds to the evidence that dental health and/or fish consumption are of great significance for CVD and that the effects may differ between stroke and AMI, as well as between fatal and non-fatal AMI. In order to better assess the protective effect of fish consumption on AMI there is a need for studies with combined information on interrelated factors of concern, e.g. usual intake of different types of fish, PUFAs, selenium and Hg, preferably based on reliable biomarkers.

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The authors declare that they have no conflicts of interest.

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Table 1. Baseline characteristics in 1968-69 in the cohort of 1462 women (percentages or medians with ranges) and Spearman´s rank correlations (r_s) with serum mercury concentrations (S-Hg).

Variable	**N**	**Median/ percent**	**Range**	**IQR**	**r_s**
S-Hg (nmol/L)	1,397	6.90	0.6- 64.5	5.1-9.3	-
Age (y)	1,397	46	38-60	38-50	-0.05
Fish intake, 24-hour recall (g)	1,235	0 (mean=26)	0-550	0-0	0.13***
Number of teeth	1,355	20 (mean=17)	0-32	9-25	0.41***
Number of amalgam surfaces	1,001	13	0-65	1-25	0.32***
Social class; high=1 (%)	1,349	1=42 2=45 3=12			0.17***
Education, more than primary school (%)	1,392	30			0.19***
Hypertension (%)	1,397	21			-0.02
Wine drinker, $\geq$1 time/month (%)	1,394	51			0.19***
Serum triglycerides (mmol/L)	1,397	1.10	0.37- 9.78	0.84-1.40	-0.14***
Serum cholesterol	1,397	6.76	3.58- 26.8	6.05-7.54	-0.02

(mmol/L)					
Waist-hip ratio	1,358	0.73	0.58- 1.00	0.70-0.77	-0.03
Diabetes (%)	1,397	0.7			-0.02
Current smoker (%)	1,396	40			-0.02

IQR, Inter-quartile range

*P<0.05, **P<0.01, ***P<0.001

Table 2. Relationships between serum mercury levels, on the one hand, and mortality and total, fatal or non-fatal acute myocardial infarction and stroke, on the other. Hazard ratio [HR (95% confidence interval)] *versus* Quartile 1. The full multivariate model includes all potential confounders (see text). N=number of cases.

Outcome	Model (adjustments)	Quartile 1	2	3	4
Mortality	Age N=501	1.00	0.85 (0.68-1.09)	0.78 (0.61-0.99)	0.76 (0.59-0.97)
	Full N=446	1.00	0.96 (0.74-1.25)	0.96 (0.73-1.24)	1.02 (0.77-1.35)
AMI Total	Age N=128	1.00	0.75 (0.47-1.20)	0.70 (0.44-1.12)	0.56 (0.34-0.93)
	Full N=116	1.00	0.85 (0.51-1.42)	1.04 (0.63-1.71)	0.83 (0.47-1.45)
Fatal	Age N=86	1.00	0.78 (0.45-1.35)	0.75 (0.43-1.27)	0.31 (0.15-0.66)
	Full N=79	1.00	0.90 (0.50-1.62)	1.02 (0.57-1.83)	0.43 (0.19-0.98)
Non-fatal	Age N=42	1.00	0.68 (0.28-1.66)	0.63 (0.26-1.54)	1.11 (0.51-2.40)
	Full N=37	1.00	0.72 (0.26-2.03)	1.05 (0.40-2.75)	1.83 (0.76-4.42)
Stroke	Age N=173	1.00	1.34 (0.86-2.08)	1.11 (0.71-1.74)	1.26 (0.81-1.97)
	Full	1.00	1.54	1.44	1.80

	N=162		(0.97-2.44)	(0.89-2.31)	(1.11-2.92)