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Cardiac autonomic activity in methylmercury neurotoxicity: 14-year follow-up of a Faroese birth cohort

Philippe Grandjean, MD, PhD, Katsuyuki Murata, MD, Esben Budtz-Jørgensen, PhD, and Pál Weihe, MD

From the Institute of Public Health, University of Southern Denmark, Odense, Denmark; the Department of Environmental Health, Harvard University School of Public Health, Boston; the Division of Environmental Health Sciences, Akita University School of Medicine, Akita, Japan; the Department of Biostatistics, Institute of Public Health, University of Copenhagen, Copenhagen, Denmark; and the Department of Occupational Medicine and Public Health, Faroese Hospital System, Tórshavn, Faroe Islands.

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Reprint requests: Philippe Grandjean, MD, Department of Environmental Health, Harvard School of Public Health, Landmark Center East room 3-110, 665, P.O.Box 15967, Boston, MA 02115.

Correspondence to: Philippe Grandjean, Institute of Public Health, University of Southern Denmark, Winsløwparken 17, 5000 Odense C, Denmark. Phone: +45-65503768. Fax: +45-65911458. Email: pgrand@hsph.harvard.edu

Key words: Electrophysiologic techniques, cardiac Environmental pollution Food contamination Methylmercury compounds Prenatal exposure delayed effects

Objective: To determine whether heart function in childhood is affected by exposure to methylmercury (MeHg) from seafood.

Study design: Prospective study of a Faroese birth cohort (N = 1,022). Examinations at ages 7 and 14 years included blood pressure, heart rate variability (HRV) and its frequency components of autonomic origin, and brainstem auditory evoked potentials (BAEP). Mercury concentrations were determined in cord blood, and in the child's hair.

Results: Both low-frequency (LF) and high-frequency (HF) activities decreased by about 25% from 7 to 14 years; they correlated well with the blood pressures. A doubling of prenatal MeHg exposure was associated with a decrease in LF and HF powers of about 6.7 % ($p = 0.04$) and in the coefficient of variation of the R-R interval of 2.7% ($p = 0.04$) at age 14 years. No discernible effect on blood pressure was apparent. Decreased LF variability was associated with increased latency of BAEP peak III, but adjustment for MeHg exposure substantially attenuated this correlation.

Conclusions: MeHg exposure was associated with decreased sympathetic (LF) and parasympathetic (HF) modulation of the HRV. Parallel MeHg-related delays of BAEP latencies may be due to underlying MeHg neurotoxicity to brainstem nuclei.

BAEP	Brainstem auditory evoked potentials
C-CVHF	Component coefficient of variance for the high-frequency band
C-CVLF	Component coefficient of variance for the low-frequency band
CVRR	Coefficient of variation for the R-R interval
HF	High frequency
HRV	Heart rate variability
LF	Low frequency
MeHg	Methylmercury

A National Research Council committee recently suggested that cardiovascular function may be vulnerable to developmental methylmercury (MeHg) exposure.¹ The evidence reviewed included a prospective birth cohort study from the Faroe Islands, where an increase in blood pressure and a decrease in the coefficient of variation for the R-R interval (CVRR) at age 7 years was observed at increased intrauterine exposure levels.² In the same children, MeHg exposure was also associated with delayed brainstem auditory evoked potential latencies³ and deficits in neuropsychological tests that reflect attention and several other functional modalities.⁴

The CVRR and its frequency components are easy to apply and highly reproducible in children.⁵⁻⁹ Sympathetic hyperactivity and parasympathetic hypoactivity have been documented in connection with cardiovascular diseases.^{6,10} The CVRR increases with age up to 6-10 years,^{11,12} followed by a decrease related to reduced parasympathetic activity.¹³ Boys have a greater CVRR than girls,¹³ and HRV is increased by physical training.¹⁴ However, very little information on children is available regarding the sensitivity of HRV to toxicants, especially those that may affect the nervous system.

In adults, HRV is sensitive to dysfunctions of autonomic tone in smokers¹⁵⁻¹⁷ and alcohol drinkers.¹⁷⁻²⁰ Abnormalities have also been documented after exposure to neurotoxic agents, such as MeHg, mercury vapor, other metals, certain solvents, and nerve gases.²¹⁻²⁸ In most cases, the CVRR was apparently affected mainly through a relative depression of parasympathetic activity.²¹ In particular, a decreased HRV in adult patients that had been born with congenital MeHg poisoning appeared to be mainly due to parasympathetic hypofunction.²⁹ Lesions located in the brainstem may affect the HRV,³⁰ and neuropathological abnormalities caused by developmental MeHg exposure include this location.³¹

In a prospective study of a birth cohort with increased intrauterine exposure to MeHg,

exposure-associated changes in CV-RR and blood pressures were recorded at age 7 years.² This population has now been re-examined at age 14 years with electrophysiologic parameters of heart function, blood pressure and evoked potentials. In addition, CVRR data from both examinations were analyzed in regard to the frequency components. It was hypothesized that MeHg exposure would affect HRV and blood pressure, and that effects, if mediated through brainstem nuclei, would be associated with known MeHg-related delays in BAEP latencies.

METHODS

Study Population and Follow-up

A cohort of 1,022 births was assembled in the Faroe Islands during a 21-month period of 1986-1987.³² The first follow-up examination was carried out seven years later and included determination of HRV and evoked potential latencies, neuropsychological testing, pediatric examination, and exposure assessment.²⁻⁴ At age 14 years, a total of 878 of 1,010 live cohort members (86.9%) were examined by comparable methods (Table I).³³ The examinations were conducted by a team of health professionals who had no access to information on individual exposure levels. The 438 boys and 440 girls examined had an average age of 13.83 (SD, 0.32) years. The two examinations involved a total of 963 cohort children (483 boys and 480 girls), of whom 813 (402 boys and 411 girls) were examined twice.

In a small number of cases, complete heart rate records were not obtained. One child had a very high systolic blood pressure and was referred to a specialist. When re-examined several weeks later, the blood pressure had substantially decreased, but matching HRV data were not obtained, and the child was therefore excluded. One child with a low birth weight² and 18 other children diagnosed with neurological or other serious diseases that may affect the nervous

system³³ were excluded. None of the children had diabetes.

At each examination, a questionnaire was filled in by a parent and provided information about past medical history and other relevant factors. Physical activity was rated much, average and none, with all children participating in such sports as soccer being rated 'much'. The pediatrician interviewed the child about smoking and use of alcohol. Birth weight, family history of hypertension / maternal hypertension (maternal hypertension risk), and smoking during pregnancy were previously recorded.² The study protocol was approved by the ethical review committee for the Faroe Islands and the Institutional Review Board at Boston University, and parental informed consent was obtained.

The primary indicator of intrauterine exposure to MeHg was the mercury concentration in cord blood; it was supplemented by the concentration in maternal hair at parturition.³² Exposure levels varied widely, with a 1,000-fold difference between the lowest and the highest mercury concentrations.³² Children participating in the follow-up had prenatal exposures similar to those recorded for the full cohort.³³ Analysis of hair obtained at the two follow-up examinations showed that the intrauterine exposure level averaged about 7-fold and 4-fold higher than the two sets of postnatal levels.³³ Results given in μg may be converted to nmol by multiplying by 5.0.

Cardiovascular Function Assessment

Two pediatricians carried out a thorough physical examination that included assessment of systolic and diastolic blood pressures thrice, and the average was calculated. With the child relaxing in a chair, a cuff that covered between 1/2 and 2/3 of the upper arm was applied on the left arm, and the pressures were read in mm Hg on a sphygmomanometer. Body weight was

measured in kg on an electronic scale to the nearest single digit after the decimal point. Standing height was measured with a stadiometer to the nearest millimeter.

At both examinations, the heart rate was measured as the average R-R interval on an electrocardiographic amplifier (NEC-Sanei 1271SP) connected to a computer.² After the child had been lying in a relaxed, supine position and breathing normally for at least 5 min, 300 R-R intervals were measured in real time (sampling time, 1 ms); 100 consecutive R-R intervals with the minimal standard deviation were automatically extracted for calculation of the average heart rate and its relative standard deviation (SD). The CVRR is the ratio of the SD of the R-R intervals to the average value (RRmean).

The cardiac sinus rhythm shows fluctuations around the mean heart rate due to continuous changes in the autonomic balance.³⁴⁻³⁶ The main periodic fluctuations consist of the respiratory sinus arrhythmia and the baroreflex-related heart rate variation at a lower frequency. Their frequencies reflect the dependence on inspiratory inhibition of the vagal tone, and the slower rhythm originating from intrinsic oscillation in the vasomotor part of the baroreflex loop. They therefore indicate parasympathetic and sympathies activities. Spectral analysis can provide estimates of the frequencies and powers (e.g., the magnitude of a cyclical component) from time-series data. We therefore applied autoregressive spectral analysis to partition the HRV into independent components.^{17,37} The results of the autoregressive spectral analysis were expressed in low frequency (LF) and high frequency (HF) components, i.e., 0.01-0.15 Hz and 0.15-0.40 Hz. As the square root of the total power spectral density is equal to the standard deviation of the R-R intervals, each component coefficient of variation (i.e., C-CVLF and C-CVHF) was defined as the ratio of the square root of each component power spectral density (PSD-LF and PSD-HF in ms^2) to the RRmean: C-CVLF (or C-CVHF) = $100 * \text{sqrt}(\text{PSD-LF (or PSD-HF)})/\text{RRmean}$. The

LF/HF power ratio indicates the balance of cardiac autonomic activity, with $LF/HF \gg 1$ and $LF/HF \ll 1$ reflecting sympathetic and parasympathetic prominence, respectively.

Of 841 subjects with HRV data at age 14, BAEPs were recorded in all but two, and in all of the 781 children who were also examined at age 7 years. A four-channel electromyograph (Medelec Sapphire-4ME) was employed as previously described.³³ Peak III latency at 20 Hz and 40 Hz at both examinations showed delays associated with intrauterine MeHg exposure.^{2,33} Peak III is thought to reflect the volume-conducted electric activity from the pons (superior olivary nucleus).³⁸

Data Analysis

Because of skewed distributions, logarithmic transformation of the contaminant concentrations was used to limit the dependence upon small numbers of children with very high exposure levels. Likewise, outcome variables other than blood pressure and heart rate required logarithmic transformation to obtain a better fit of the regression models. For transformed variables, geometric means were supplemented by interquartile ranges (25th and 75th percentiles). Pearson's correlation coefficients were used to assess bivariate relationships, and adjustment for confounders was included in partial correlation coefficients. Differences between paired examination data were assessed by matched-pairs t test, while those between sexes were analyzed by unpaired t-test.

Regression analysis was carried out to determine the association between MeHg exposure and the outcome variables. Birth weight, maternal hypertension risk and maternal smoking during pregnancy, and the child's age, sex, height and weight were included as confounders. Tanner stage was assessed at age 14 years but was not included because of the

close association with age and anthropometric parameters, and because this variable was missing from several subjects. Additional covariates at age 14 years were regular smoking (information was missing from 31 subjects), and physical activity rated 'much'. Blood pressure was adjusted for examiner.

For log transformed outcome variables, the MeHg regression coefficients were modified to indicate the relative change (in percent) of the average of the dependent variable associated with a doubling of the MeHg exposure. The heart rate was subsequently added in models where other outcomes were used as effect parameters. To determine whether changes between the two examinations were associated with MeHg exposure, the paired result obtained seven years before was introduced as predictor of the outcome at age 14 years. Potentially nonlinear exposure-effect relationships² were again explored in generalized additive models, which do not require linearity assumptions while providing a smooth non-parametric dose-response curve.³⁹

RESULTS

HRV Changed with Age but Remained a Significant Predictor of Blood Pressure

Paired HRV results from ages 7 and 14 years showed substantial changes between the two examinations, with decreases of about 25% in both low frequency (LF) and high frequency (HF) powers and their variabilities (Table II). However, the paired data on heart rate, CVRR, and the HF power correlated well. Boys and girls generally had similar results, except for a higher heart rate in girls at 7 years and a higher systolic blood pressure in boys at 14 years.

A lower CVRR, a lower HF power, and a higher LF / HF ratio were significant predictors of increased systolic blood pressure at both examinations, and a lower LF power was also significant at age 7 (Table III). Diastolic blood pressure was only weakly related to the HRV

parameters.

Both LF and HF Decreased at Higher MeHg Exposures

Adjusted regression coefficients showed significant negative impacts of MeHg on several parameters (Table IV). Both LF and HF decreased at higher prenatal MeHg exposure. Postnatal exposure at age 7 years seemed mainly related to LF, while mainly to HF at age 14 years. Inclusion of more than one MeHg exposure indicator as independent variables resulted in attenuated MeHg regression coefficients in the same direction. The correlation between prenatal and postnatal exposures (correlation coefficients of 0.33 and 0.35)³³ therefore did not allow a clear separation of the impact of exposures at different developmental stages.

A non-linear association between prenatal MeHg exposure and blood pressure and CVRR was previously found at age 7 years, where the strongest effect was seen at cord-blood concentrations below 10 g/L.² This association was again explored using generalized additive models. Fig 1 shows that, while significant at age 7 years,² the effect on blood pressure is no longer significant at age 14 years. However, similar modeling of CVRR showed that an effect of prenatal MeHg exposure is clearly present also at age 14 years (Fig 2). While the change in CVRR within the low-level exposure range below 10 g/L was not statistically significant, the heart rate increased by 2.67 (95% CI, 0.08; 5.27) for each doubling in MeHg exposure within that interval. Heart rate was associated with almost all outcomes, but adjustment for this variable resulted in only marginal changes of the MeHg regression coefficients. However, when the previous outcome at age 7 years was included as an additional independent variable, the MeHg effects on the same outcome at 14 years was attenuated and became non-significant.

LF Power Was Associated with BAEP Latencies

To ascertain possible associations with brainstem functions, correlations were calculated

with the latency of BAEP peak III, which appears to be increased by prenatal MeHg exposure.^{10,33}

The LF power and its component CV showed clear negative associations with peak III latencies, viz., the greater the BAEP latency, the less the LF power and its CV (Table V). To determine whether these associations were innate, partial correlation coefficients were calculated with adjustment for prenatal and postnatal MeHg exposure biomarkers. These adjusted correlations were substantially attenuated and tended to lose statistical significance.

DISCUSSION

This prospective study of a birth cohort provides information on developmental changes in cardiac autonomic activity and on the impact of MeHg neurotoxicity. A major advantage of the present study is that birth cohort members were examined prospectively first at school age and then at early adolescence. Maturation of the cardiac autonomic activity results in an increase in the CVRR with gestational age and during early postnatal life,³⁴ then followed by a decline of CVRR as well as in C-CVLF and C-CVHF. Infants particularly have a high sympathetic activity that then decreases between ages 5 and 10 years, while the sympatho-vagal balance as expressed by the LF / HF ratio changes less.¹² The age-related decreases in heart rate, CVRR, C-CVHF, and HF power now documented are in accordance with the expectation from smaller, cross-sectional studies.^{9,11-13} Despite maturation changes, the CVRR and HF results at the two examinations correlated well, although the LF results varied more. The blood pressure levels and the age-associated increase in systolic blood pressure are in accordance with data from previous cross-sectional studies.⁴⁰ Only limited sex-related differences were seen at the two ages examined, while some,^{9,12} but not all,^{17,21} previous studies reported an HRV difference between small numbers of boys and girls. An increased LF / HF ratio is thought to act as a predictor of

increased blood pressure,¹⁰ and the results of the present study support this notion by the significant associations between increased systolic blood pressure and decreases in CVRR and HF results. Conversely, physical training has been reported decrease blood pressures and increase both LF and HF powers.¹⁴ Again, the present results are in agreement with previous research.

Only sparse information is available on adverse effects on HRV in children, e.g., following developmental exposure to neurotoxicants. The paucity of information is perhaps not surprising, given the substantial age-dependency of the HRV parameters that might complicate interpretation of cross-sectional studies. Studies of adults with occupational exposure to neurotoxicants²¹⁻²⁸ clearly show that HRV may be affected by chemical exposures that damage the nervous system. However, effects on children would be difficult to predict from studies conducted in adults.

A central origin of autonomic oscillations of the HRV is indicated by experimental data⁴¹ and by decreases in HRV parameters in relation to central damage of the brainstem nuclei associated with autonomic nervous function.³⁰ In a study of lead-exposed workers, electrophysiological parameters showed a significant exposure-associated HRV depression, although not the anticipated delayed latencies of BAEP peaks.²⁴ This finding may suggest that changes in HRV could be an earlier effect than increased BAEP latencies. Another study of occupational lead exposure reported an association between lead-induced changes in HRV and peripheral nerve conduction.⁴²

The impact of prenatal MeHg poisoning on HRV has recently been documented in survivors who had reached adult age.²⁹ Although only a small number of patients was examined, a significant decrease in HF results was reported as a lasting abnormality. The present study

deals with a much larger number of subjects exposed to much lower levels of MeHg from contaminated marine food. At both examinations, both HF and LF components appeared to be affected, but the effect on LF seemed to be less at age 7 years. A difference in sensitivity of HRV components could be due to normal age-related changes in cardiac autonomic activity. Despite significant associations between exposure indicators and several HRV outcomes, a MeHg effect on blood pressure previously documented at age 7 years² was not discernible at age 14 years. Also, the significant associations of 14-year outcomes with prenatal MeHg exposure were attenuated after adjustment for the 7-year outcomes. Thus, the developmental MeHg exposure did not result in substantial further changes beyond those observed at age 7 years.

The plausibility of the findings reported here is supported by the strong evidence of MeHg neurotoxicity.^{1,29,31} Children with acute mercury vapor poisoning often have increased heart rate and increased blood pressure.⁴³ In rats exposed to high doses of MeHg chloride, changes in normal heart rate variations were induced,⁴⁴ and an increase in systolic blood pressure seen in another study persisted for at least 9 months.⁴⁵

A link between brainstem functions and autonomic tone is supported by the associations in the present study between BAEP latencies and HRV parameters, especially the LF results. Although only weak and mostly non-significant after adjustment for MeHg exposure, the possibility cannot be completely discounted that such correlations are normal and unrelated to neurotoxicant exposures. However, the fact that MeHg affects both parameters would suggest that the exposure-related changes in HRV at least in part reflect MeHg neurotoxicity exerted in the brainstem nuclei. In addition, the existence of neurotoxic effects on the brainstem is suggested by highly significant MeHg-associated deficits in neuropsychological tests of attention.⁴ This hypothesis therefore deserves to be examined in experimental studies.

While HRV parameters are highly reproducible under standardized conditions,⁵⁻⁹ a possible limitation of the present study is that HRV was assessed only during a brief period, and that respiratory patterns were not controlled. Respiratory activity may have affected the HF component,^{34,35} but the children examined were resting in a darkened room, and the 100-heart-beat sequence with the lowest variation was selected for statistical analysis. Also, the time of the testing, relation to meals and exercise, temperature of the laboratory, etc., have limited effects on HRV results.³⁵ Some circadian variation has been described,¹³ but the cohort children were all examined between 8 a.m. and 5 p.m., where the variation is expected to be the least. While some HRV results may be positively associated with heart rate,¹⁷ adjustment for this variable affected the MeHg regression coefficients only slightly. Also, by examining the CVRR rather than the RR itself, an adjustment for dependence on heart rate was included. As mentioned above, age is known to affect HRV results, but at the time of each examination, the subjects were of virtually the same age, and both age and sex were incorporated as mandatory covariates in the statistical analyses. At age 14 years, Tanner stage was not an important predictor, but rapid changes in height, weight, and other developmental factors at puberty age may have decreased the sensitivity of the study in identifying puberty-related effects on, e.g., blood pressure. CVRR and other HRV parameters may be affected by alcohol and smoking habits,¹⁵⁻²⁰ but few of the subjects examined had used tobacco and alcohol, and adjustment for smoking was included as a covariate. While several diseases, such as congenital heart disease⁶ and asthma,⁸ are known to affect the CVRR, this cohort was population-based, and children with relevant diagnoses were excluded.

An important short-coming is that MeHg exposure was assessed only at three points in time, and that correlations between the three measures prevented distinction between effects of prenatal and postnatal exposures. Most likely, the three biomarkers are imprecise indicators of

the MeHg concentrations that may have caused the effects during the 14-year lifespan of the children. Such non-differential misclassification is likely to bias the finding toward the null hypothesis. Thus, the present study has the advantage of size, prospective follow-up, and adjustment for relevant confounders. However, imprecision, especially of the exposure assessment would tend to cause an underestimation of the true MeHg effects.

Clinical studies of adults have reported that a decreased cardiac vagal tone is associated with an increased risk of sudden cardiac death or coronary artery disease, and measurements of HRV and the quantification of its spectral components are therefore considered powerful predictors of cardiovascular morbidity and mortality.³⁵ Recent findings in adults suggest that MeHg exposure is associated with increased cardiovascular mortality.^{46,47} Although several toxic mechanisms may be involved, these findings in conjunction with the present study suggest that the impact of neurotoxic MeHg effects on autonomic regulation of heart function deserves attention.

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REFERENCES

1. National Research Council. Toxicological Effects of Methylmercury. Washington, DC: National Academy Press; 2000.
2. Sørensen N, Murata K, Budtz-Jørgensen E, Weihe P, Grandjean P. Prenatal methylmercury exposure as a cardiovascular risk factor at seven years of age. *Epidemiol* 1999; 10: 370-5.
3. Murata K, Weihe P, Araki S, Budtz-Jørgensen E, Grandjean P. Evoked potentials in Faroese

- children prenatally exposed to methylmercury. *Neurotoxicol Teratol* 1999; 21: 471-2.
4. Grandjean P, Weihe P, White RF, Debes F, Araki S, Murata K, et al. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicol Teratol* 1997; 19: 417-28.
 5. Massin M, von Bernuth G. Normal ranges of heart rate variability during infancy and childhood. *Pediatr Cardiol* 1997; 18: 297-302.
 6. Massin MM, Derkenne B, von Bernuth G. Correlations between indices of heart rate variability in healthy children and children with congenital heart disease. *Cardiology* 1999; 91: 109-13.
 7. Batten LA, Urbina EM, Berenson GS. Interobserver reproducibility of heart rate variability in children (the Bogalusa Heart Study). *Am J Cardiol* 2000; 86:1264-6.
 8. Kazuma N, Otsuka K, Miyakawa M, Shirase E, Matsuoka I, Murata M. Seasonal variation in heart rate variability in asthmatic children. *Chronobiol Int* 2000; 17: 503-11.
 9. Silveti MS, Drago F, Ragonese P. Heart rate variability in healthy children and adolescents is partially related to age and gender. *Int J Cardiol* 2001; 81: 169-74.
 10. Stewart JM. Does heart rate variability explain increased blood pressure in adolescents? *J Pediatr* 2000; 137: 6-8.
 11. Finley JP, Nugent ST. Heart rate variability in infants, children and young adults. *J Auton Nerv Syst* 1995; 51: 103-8.
 12. Goto M, Nagashima M, Baba R, Nagano Y, Yokota M, Nishibata K, Tsuji A. Analysis of heart rate variability demonstrates effects of development on vagal modulation of heart rate in healthy children. *J Pediatr* 1997; 130: 725-9.
 13. Otsuka K, Cornelissen G, Halberg F. Age, gender and fractal scaling in heart rate variability.

- Clin Sci (Lond) 1997; 93: 299-308.
14. Mandigout S, Melin A, Fauchier L, N'Guyen LD, Courteix D, Obert P. Physical training increases heart rate variability in healthy prepubertal children. *Eur J Clin Invest* 2002; 32: 479-87.
 15. Robertson D, Tseng CJ, Appalsamy M. Smoking and mechanisms of cardiovascular control. *Am Heart J* 1988; 115: 258-63.
 16. Hayano J, Yamada M, Sakakibara Y, Fujinami T, Yokoyama K, Watanabe Y, Takata K. Short- and long-term effects of cigarette smoking on heart rate variability. *Am J Cardiol* 1990; 65: 84-8.
 17. Murata K, Landrigan PJ, Araki S. Effects of age, heart rate, gender, tobacco and alcohol ingestion on R-R interval variability in human ECG. *J Auton Nerv Syst* 1992; 37: 199-206.
 18. Weise F, Krell D, Brinkhoff N. Acute alcohol ingestion reduces heart rate variability. *Drug Alcohol Depend* 1986; 17: 89-91.
 19. Yokoyama A, Takagi T, Ishii H, Muramatsu T, Akai J, Kato S, et al. Impaired autonomic nervous system in alcoholics assessed by heart rate variation. *Alcohol Clin Exp Res* 1991; 15: 761-5.
 20. Murata K, Araki S, Yokoyama K, Sata F, Yamashita K, Ono Y. Autonomic neurotoxicity of alcohol assessed by heart rate variability. *J Auton Nerv Syst* 1994; 48: 105-11.
 21. Araki S, Murata K, Yokoyama K. Application of neurophysiological methods in occupational medicine in relation to psychological performance. *Ann Acad Med Singapore* 1994; 23: 710-8.
 22. Murata K, Araki S, Yokoyama K, Maeda K. Autonomic and peripheral nervous system dysfunction in workers exposed to mixed organic solvents. *Int Arch Occup Environ Health*

- 1991; 63: 335-40.
23. Murata K, Araki S, Yokoyama K. Assessment of the peripheral, central and autonomic nervous system function in styrene workers. *Am J Ind Med* 1991; 20: 775-84.
 24. Murata K, Araki S, Yokoyama K, Nomiyama K, Nomiyama H, Tao YX, et al. Autonomic and central nervous system effects of lead in female glass workers in China. *Am J Ind Med* 1995; 28: 233-44.
 25. Murata K, Araki S, Yokoyama K, Okumura T, Ishimatsu S, Takasu N, et al. Asymptomatic sequelae to acute sarin poisoning in the central and autonomic nervous system 6 months after the Tokyo subway attack. *J Neurol.* 1997; 244: 601-6.
 26. Magari SR, Schwartz J, Williams PL, Hauser R, Smith TJ, Christiani DC. The association of particulate air metal concentrations with heart rate variability. *Environ Health Perspect* 2002; 110: 875-80.
 27. Bockelmann I, Pfister EA, McGauran N, Robra BP. Assessing the suitability of cross-sectional and longitudinal cardiac rhythm tests with regard to identifying effects of occupational chronic lead exposure. *J Occup Environ Med* 2002; 44: 59-65.
 28. Jhun HJ, Yim SH, Kim R, Paek D. Heart-rate variability of carbon disulfide-poisoned subjects in Korea. *Int Arch Occup Environ Health* 2003; 76: 156-60.
 29. Oka T, Matsukura M, Okamoto M, Harada N, Kitano T, Miike T, et al. Autonomic nervous functions in fetal type Minamata disease patients: assessment of heart rate variability. *Tohoku J Exp Med* 2003; 198: 215-21.
 30. Shimomura C, Matsuzaka T, Koide E, Kinoshita S, Ono Y, Tsuji Y, et al. Spectral analysis of heart rate variability in the dysfunction of brainstem. *Brain Development* 1991; 23: 26-31 (In Japanese).

31. Takeuchi T, Eto K. *The Pathology of Minamata Disease. A Tragic Story of Water Pollution.* Fukuoka: Kyushu University Press, 1999.
32. Grandjean P, Weihe P, Jørgensen PJ, Clarkson T, Cernichiari E, Viderø T. Impact of maternal seafood diet on fetal exposure to mercury, selenium, and lead. *Arch Environ Health* 1992; 47: 185-95.
33. Murata K, Weihe P, Budtz-Jørgensen E, Jørgensen PJ, Grandjean P. Delayed brainstem auditory evoked potential latencies in 14-year-old children exposed to methylmercury. *J Pediatr* (submitted).
34. van Ravenswaaij-Arts CMA, Kollee LAA, Hopman JCW, Stoelinga GBA, van Geijn HP. Heart rate variability. *Ann Int Med* 1993; 118: 436-47.
35. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circulation* 1996; 93: 1043-65.
36. Murata K, Araki S. Assessment of autonomic neurotoxicity in occupational and environmental health as determined by ECG R-R interval variability: a review. *Am J Ind Med* 1996; 30: 155-163.
37. Akselrod S. Spectral analysis of fluctuations in heart rate and other cardiovascular parameters as a tool for the assessment of autonomic control. In: Korczyn AD, editor. *Handbook of Autonomic Nervous System Dysfunction.* New York: Marcel Dekker, 1995, p. 469-93.
38. Chiappa KH, Hill RA. Brainstem auditory evoked potentials: interpretation. In: Chiappa KH, editor. *Evoked potentials in clinical medicine*, 3rd ed. Philadelphia, PA: Lippincott-Raven, 1997, p. 199-268.

39. Hastie TJ, Tibshirani RJ. *Generalized Additive Models*. Boca Raton: CRC Press, 1990.
40. Brotons C, Singh P, Nishio T, Labarthe DR. Blood pressure by age in childhood and adolescence: a review of 129 surveys worldwide. *Int J Epidemiol* 1989; 18: 824-9.
41. Grasso R, Rizzi G, Schena F, Cevese A. Arterial baroreceptors are not essential for low frequency oscillation of arterial pressure. *J Auton Nerv Syst*. 1995; 50: 323-31.
42. Murata K, Araki S. Autonomic nervous system dysfunction in workers exposed to lead, zinc, and copper in relation to peripheral nerve conduction: a study of R-R interval variability. *Am J Ind Med* 1991; 20: 663-71.
43. Warkany J, Hubbard DM. Acrodynia and mercury. *J Pediatr* 1953; 42: 365-86.
44. Arito H, Takahashi M. Effect of methylmercury on sleep patterns in the rat. In: Suzuki T, Imura N, Clarkson TW, editors. *Advances in Mercury Toxicology*. New York: Plenum, 1991, p. 381-94.
45. Wakita Y. Hypertension induced by methyl mercury in rats. *Toxicol Appl Pharmacol* 1987; 89: 144-7.
46. Salonen JT, Seppanen K, Nyysönen K, Korpola H, Kauhanen J, Kantola M, Tuomilehto J, Esterbauer H, Tatzber F, Salonen R. Intake of mercury from fish, lipid peroxidation, and the risk of myocardial infarction and coronary, cardiovascular, and any death in eastern Finnish men. *Circulation* 1995; 91: 645-55.
47. 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: 1747-54.

Table I. Characteristics of 424 boys and 433 girls from the Faroese birth cohort examined at age 14 years

Variable	
Maternal age in years	27.4 (5.4)
Previous births (none / one / at least two in %)	33.4 / 34.5 / 32.1
Smoking during pregnancy (no / yes in %)	59.3 / 40.7
Alcohol consumption during pregnancy (never / ever in %)	76.5 / 23.5
Gestational age in weeks	40.3 (1.3)
Birth weight in g	3680 (528)
Age at 7-year examination in years*	6.84 (0.31)
Body weight in kg*	24.45 (3.85)
Height in cm*	122.4 (5.07)
Age at 14-year examination in years	13.83 (0.32)
Year of examination (% in 2000 / 2001)	55.8 / 44.2
Body weight in kg	54.58 (11.20)
Height in cm	162.9 (7.26)
Smoking (no / yes in %)	94.6 / 5.4

Data for continuous variables are given as mean (SD).

*Data for 401 boys and 411 girls who were also examined at age 7 years

Table II. Cardiovascular function results in boys and girls at 7 years and 14 years after exclusion of children with relevant diagnoses. For blood pressures and heart rate, the results are given as mean (SD). For the other outcomes, data are given as geometric mean (interquartile range).

Parameter	7 years		14 years		Correlation coefficient [§]
	Boys	Girls	Boys	Girls	
Systolic blood pressure (mm Hg)	100.2 (8.02)*	100.0 (8.57)*	115.5 (8.27) [†]	113.0 (7.88)	0.319
Diastolic blood pressure (mm Hg)	64.7 (8.63)	65.0 (8.38)	63.1 (8.45)	64.1 (7.80)	0.194
Heart rate (min ⁻¹)	80.7 (9.52)* [†]	85.0 (10.93)*	69.6 (10.86)	71.5 (10.96)	0.437
CVRR (%)	7.62 (5.17-11.53)*	7.93 (5.55-11.42)*	5.91 (4.26-8.17)	6.16 (4.58-8.25)	0.406
LF power (n.u.)	954 (481-2130)	1076 (475-2457)*	829 (413-1833)	849 (439-1796)	0.249
HF power (n.u.)	1779 (807-5270)*	1787 (709-5025)*	1252 (578-2826)	1439 (644-3312)	0.406
LF / HF	0.539 (0.273-1.007)	0.603 (0.302-1.232)	0.663 (0.343-1.341)	0.590 (0.320-1.101)	0.190
C-CVLF	4.13 (2.96-5.99)*	4.61 (3.18-6.57)*	3.31 (2.39-4.86)	3.43 (2.51-4.99)	0.210
C-CVHF	5.64 (3.78-8.97)*	5.94 (4.11-9.25)*	4.06 (2.89-5.88)	4.47 (3.26-6.09)	0.374

*p < 0.01 for difference between outcome at ages 7 and 14 years for fixed sex; [†]p < 0.01 for difference between outcome in boys and girls at the same age; [§]Partial correlation coefficient (after logarithmic transformation of HRV parameters) with adjustment for sex, blood pressure also adjusted for examiner (p < 0.0001)

Table III. Heart rate variability parameters (log transformed) as predictors of blood pressures in Faroese cohort members at 7-year and 14-year examinations. Results are given as partial correlation coefficients adjusted for sex and (at age 14 years) examiner (*P* value).

Parameter	7 years		14 years	
	Systolic	Diastolic	Systolic	Diastolic
CVRR	-0.12 (<0.001)	-0.09 (0.012)	-0.07 (0.030)	-0.05 (0.175)
LF power	-0.09 (0.006)	-0.07 (0.040)	-0.03 (0.427)	-0.03 (0.420)
HF power	-0.13 (<0.001)	-0.07 (0.051)	-0.13 (<0.001)	-0.06 (0.072)
LF / HF	0.07 (0.031)	0.01 (0.842)	0.12 (<0.001)	0.04 (0.242)
C-CVLF	-0.07 (0.040)	-0.05 (0.117)	0.01 (0.800)	0.00 (0.944)
C-CVHF	-0.12 (<0.001)	-0.06 (0.096)	-0.11 (0.002)	-0.04 (0.292)

Table IV. Estimated effects of a doubling of MeHg exposure as indicated by three different exposure biomarkers after adjustment for confounders (p value). For log transformed HRV outcomes, the effect is expressed as the relative change (%) in the untransformed variable.

Outcome	Cord blood	Hair at 7 yrs	Hair at 14 yrs
7 years			
Heart rate (min ⁻¹)	0.250 (0.38)	-0.029 (0.90)	-
CVRR*	-1.27 (0.36)	-0.344 (0.76)	-
LF power*	-5.98 (0.047)	-5.13 (0.041)	-
HF power*	-5.16 (0.19)	-0.179 (0.96)	-
LF / HF*	-0.860 (0.79)	-4.95 (0.055)	-
C-CVLF*	-2.81 (0.052)	-2.67 (0.025)	-
C-CVHF*	-2.38 (0.19)	-0.159 (0.92)	-
14 years			
Systolic blood pressure (mm Hg)	0.045 (0.84)	0.040 (0.83)	-0.017 (0.91)
Diastolic blood pressure (mm Hg)	0.121 (0.60)	0.018 (0.93)	-0.086 (0.61)
Heart rate (min ⁻¹)	0.355 (0.26)	0.393 (0.13)	0.408 (0.073)
CVRR*	-2.73 (0.035)	-1.12 (0.30)	-1.52 (0.11)
LF power*	-6.70 (0.038)	-2.97 (0.27)	-2.06 (0.40)
HF power*	-6.78 (0.038)	-4.70 (0.088)	-4.61 (0.056)
LF / HF*	0.112 (0.97)	1.82 (0.45)	2.69 (0.21)
C-CVLF*	-2.90 (0.070)	-0.978 (0.47)	-0.483 (0.68)
C-CVHF*	-2.95 (0.046)	-1.87 (0.13)	-1.79 (0.099)

* Log transformed

Table V. Sex-adjusted partial correlation coefficients (p value) for paired results of brainstem auditory evoked potential peak latency III in relation to cardiovascular parameters at the 14-year examination, with blood pressures also adjusted for examiner, before and after additional adjustment for mercury exposure.

Outcome	Sex adjusted	Adjusted also for mercury
Systolic blood pressure	-0.05 (0.14)	-0.04 (0.30)
Diastolic blood pressure	-0.01 (0.82)	-0.04 (0.26)
Heart rate	-0.09 (0.007)	-0.07 (0.042)
CVRR*	-0.01 (0.67)	0.00 (0.95)
LF power*	-0.06 (0.073)	-0.04 (0.33)
HF power*	0.03 (0.37)	0.03 (0.43)
LF / HF*	-0.11 (0.002)	-0.07 (0.040)
C-CVLF*	-0.09 (0.009)	-0.06 (0.11)
C-CVHF*	0.01 (0.86)	0.01 (0.79)

* Log transformed

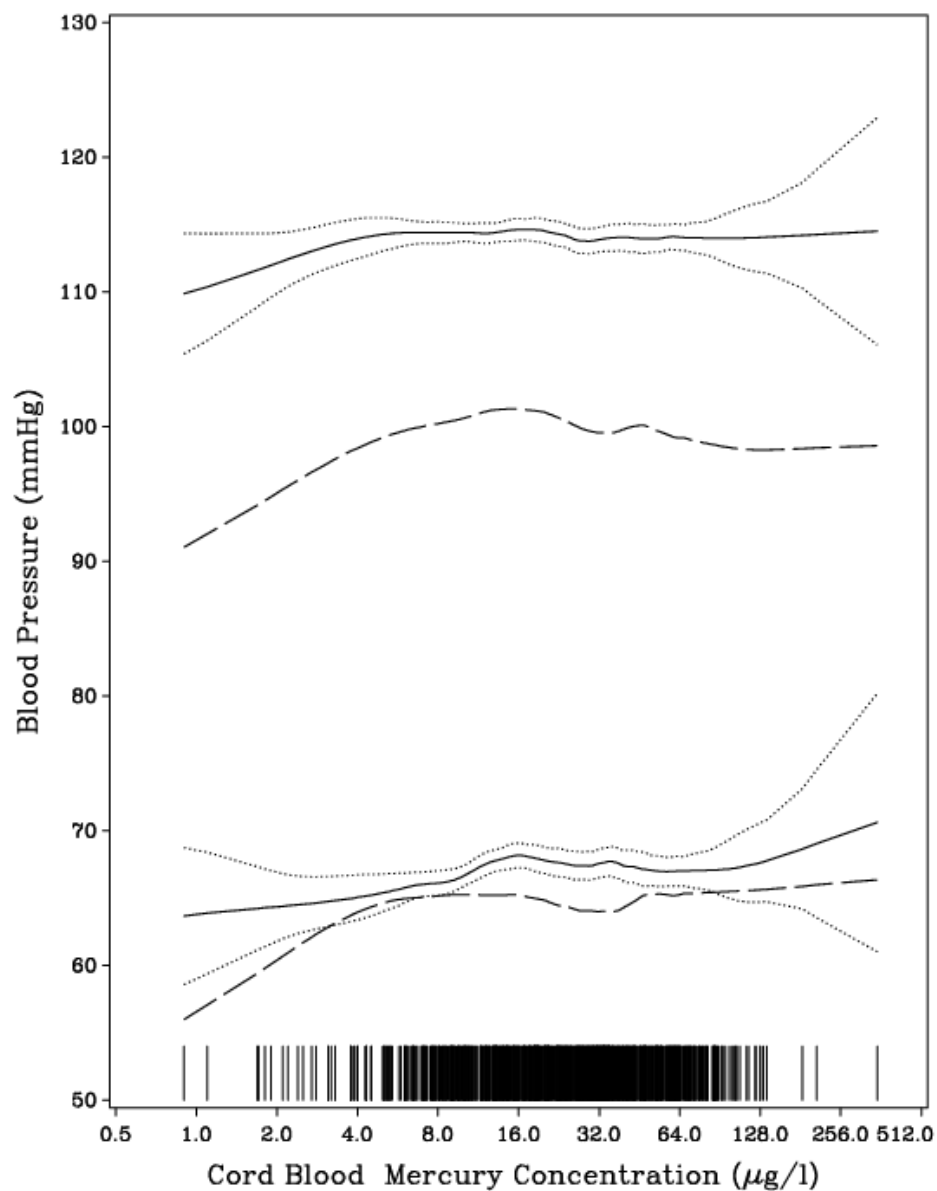


Fig 1. Generalized additive model for prenatal MeHg exposure (indicated by the mercury concentration in cord blood) as predictor of blood pressures at ages 7 and 14 years after adjustment for confounders. Broken lines depict the averages at age 7, while dotted lines indicate 95% confidence limits for the 14-year results.

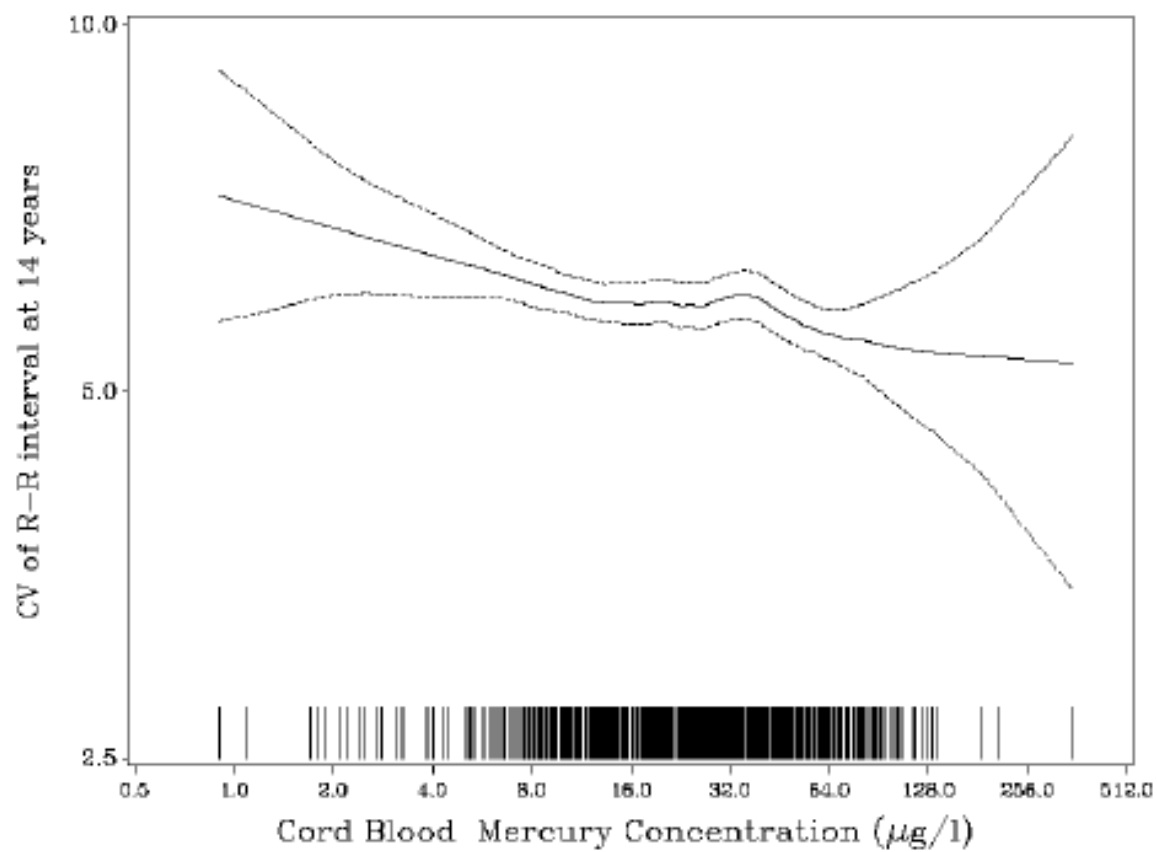


Fig 2. Generalized additive model with 95% confidence limits for prenatal MeHg exposure (indicated by the mercury concentration in cord blood) as predictor of the CVRR at 14 years of age after adjustment for confounders.