be paid to gestation rather than the breast-feeding period to avoid dangers of MeHg to human infants. Further, if exposure levels are constant and low enough not to cause adverse effects on fetuses during gestation, mothers need not worry about breast-feeding. The benefits of breast-feeding (Oddy, 2001; Kunz et al., 1999) may well outright the possible adverse effects of MeHg in breast milk under these conditions. However, if mothers were exposed to high MeHg levels during the suckling period, due caution is recommended concerning breast-feeding as the possible poisoning was reported in Iraq (Amin-Zaki et al., 1981). In addition, attention should be paid to MeHg exposure through fish and sea mammals as baby food during breast-feeding period and after weaning.

References

1. Akagi, H., Grandjean, P., Takizawa, Y. and Weihe, P. : Methylmercury dose estimation from umbilical cord concentrations in patients with Minamata disease. Environ. Res., 77(2), 98-103, 1998.
2. Amin-Zaki, L., Elhassani, S., Majeed, M.A., Clarkson, T.W., Doherty, R.A. and Greenwood, M. : Intra-uterine methylmercury poisoning in Iraq. Pediatrics. 54, 587-595, 1974.
3. Amin-zaki, L., Majeed, M.A., Greenwood, M.R., Elhassani, S.B., Clarkson, T.W.R. and Doherry, A. : Methylmercury poisoning in the Iraqi suckling infant: a longitudinal study over five years. J. Appl. Exp. Toxicol., 1, 210-214, 1981.
4. Ashner, M. and Clarkson, T.W. : Diatribution of mercury 203 in pregnant rats and their fetuses following systematic infusion with thiolcontaining amino acids and glutathione during late gestation. Teratology, 38, 145-155, 1988.
5. Cernichiari, E., Brewer, R., Myers, G.J., Marsh, D.O., Lapham, L.W., Cox, C., Shamlaye, C.F., Berlin, M., Davidson, P.W. and Clarkson, T.W. : Monitoring methylmercury during pregnancy: maternal hair predicts fetal brain exposure. Neurotoxicology. 16, 705-710, 1995.
6. Choi, B.H. : The effects of methylmercury on the developing brain. Prog. Neurobiol., 321, 447-470, 1989.
7. Clarkson, T.W. and Strain, J.J. : Nutritional factors may modify the toxic action of methyl mercury in fish-eating populations. J. Nutr, 133, 1539-1543, 2003.
8. Eto, K., Yasutake, A., Miyamoto, K., Tokunaga, H. and Otsuka, Y. : Chronic effects of methylmercury in rats. II. Pathological aspects. Tohoku J. Exp. Med., 182, 197-205, 1997.

9. Furst, A. : Can nutrition affect chemical toxicity? Int. J. Toxicol., 21, 419-424, 2002.
10. Galster, W.A. : Mercury in Alaskan Eskimo mothers and infants. Environ. Health Perspect. 15, 135-140, 1976.
11. Grandjean, P., Jørgensen, P.J. and Weihe, P. : Human milk as a source of methylmercury exposure in infants. Environ. Health Perspect., 102, 74-77, 1994.
12. Hansen, J.C., Tarp, U. and Bohm, J. : Prenatal exposure to methylmercury among Greenlandic polar Inuits. Arch. Environ. Health, 45, 355-358, 1990.
13. Kajiwara, Y., Yasutake, A., Adachi, T. and Hirayama, K. : Methylmercury transport across the placenta via neutral amino acid carrier. Arch. Toxicol., 70, 310-314, 1996.
14. Kershaw, T.G., Clarkson, T.W. and Dhahir, P.H. : The relationship between blood levels and dose of methylmercury in man. Arch. Environ. Health, 35, 28-36, 1980.
15. Kosta, L., Byrne, A.R. and Dermelj, M. : Trace elements in some human milk samples by radiochemical neutron activated analysis. Sci. Total Environ., 29, 261-268, 1982.
16. Kunz, C., Rodriguez-Palmero, M., Koletzko, B. and Jensen, R. : Nutritional and biochemical properties of human milk, Part 1: General aspects, proteins, and carbohydrates. Clin. Perinatol., 26, 307-333, 1999.
17. Newland, M.C. and Reile, P.A. : Blood and brain mercury levels after chronic gestational exposure to methylmercury in rats. Toxicol. Sci., 50, 101-116, 1999.
18. Nunes, J. and Ferreira, S. : Possible mercury intoxication of newborns via breast milk. Front. Fetl. Health, 2, 18-20, 2000.
19. Oddy, W.H. : Breastfeeding protects against illness and infection in infants and children. Breastfeed Rev., 9, 11-18, 2001.
20. Oliveira, R.B., Malm, O. and Guimarães, J.R.D. : Distribution of methylmercury and inorganic mercury in neonate hamsters dosed with methylmercury during fetal life. Environ. Res. Section A, 86, 73-79, 2001.
21. Oskarsson, A., Hallén, I.P. and Sundberg, J. : Exposure to toxic elements via breast milk. Analyst., 120, 765-770, 1995.
22. Oskarsson, A., Schts, A., Skerfving, S., Hallén, I.P., Ohlin, B. and Lagerkvist, B.J. : Total and inorganic mercury in breast milk and blood in relation to fish consumption and amalgam fillings in lactating women. Arch. Environ. Health, 51, 234-241, 1996.
23. Pan, H.S., Sakamoto, M., Oliveira, R.B., Liu, X.J., Kakita, A. and Futatsuka, M. : Changes in methylmercury accumulation in the brain of rat offspring throughout gestation and suckling. Toxicol. Environ. Chem., 86, 161-168, 2004.

24. Rice, D.C. and Barone, S. : Critical periods of vulnerability for developing nervous system: evidence from human and animal models. *Environ. Health Perspect.*, **108**, 511-533, 2000.

25. Sakamoto, M., Kakita, A., Wakabayashi, K., Nakano, A., Takahashi, H. and Akagi, H. : Evaluation of changes in methylmercury accumulation in the developing rat brain and its effects: a study with consecutive and moderate dose exposure through gestation and lactation periods. *Brain Res.*, **949**, 51-59, 2002a.

26. Sakamoto, M., Kubota, M., Matsumoto, S., Nakano, A. and Akagi, H. : Declining risk of methylmercury exposure to infants during lactation. *Environ. Res.*, **90**, 185-189, 2002b.

27. Sakamoto, M., Nakano, A., Akagi, H., Kitano, T. and Futatuka, M. : Difference by sex and age of mercury concentration in red blood cells. *Jpn. J. Hyg.* **48**, 911-919, 1993a.

28. Sakamoto, M., Nakano, A., Kajiwara, Y., Naruse, I. and Fujisaki, T. : Effects of methylmercury in postnatal developing rats. *Environ. Res.*, **61**, 43-50, 1993b.

29. Sandborgh-Englund, G., Ask, K., Belfrage, E. and Ekstrand, J. : Mercury exposure in utero and during infancy. *J. Toxicol. Environ. Health*, **63**, 317-320, 2001.

30. Skerfving, S. : Mercury in women exposed to methylmercury through fish consumption, and in their newborn babies and breast milk. *Bull. Environ. Contam. Toxicol.*, **41**, 475-482, 1988.

31. Sundberg, J., Jönsson, S., Karlsson, M.O. and Oskarsson, A. : Lactation exposure and neonatal kinetics of methylmercury and inorganic mercury in mice. *Toxicol. Appl. Pharmacol.*, **154**, 160-169, 1999.

32. Sundberg, J., Oskarsson, A. and Albanus, L. : Methylmercury exposure during lactation: milk concentration and tissue uptake of mercury in the neonate rats. *Bull. Environ. Contam. Toxicol.*, **46**, 255-262, 1991.

33. Suzuki, T., Takemoto, T., Shimano, H., Miyama, Y., Katsunuma, H. and Kagawa, Y. : Mercury content in the blood in relation to dietary habit of the women without any occupational exposure to mercury. *Ind. Health*, **9**, 1-8, 1971.

34. Svensson, B.G., Schüllts, A., Nilsson, A., Åkesson, I., Åkesson, B. and Skerfvong, S. : Fish as a source of exposure to mercury and selenium. *Sci. Total Environ.*, **126**, 61-74, 1992.

35. Swedish Expert Group : Mercury in fish: A toxicological-epidemiologic evaluation of risks. *Nord Hygienisk Tidskrift*, Suppl. 4. Stockholm, Sweden, 1971.

36. Takeuchi, T. : Pathology of Minamata disease, with special reference to its pathogenesis. *Acta Pathol. Jpn.*, **32**(Suppl. 1), 73-99, 1982.

37. World Health Organization (Eds.) : IPCS. *Environmental Health Criteria 101. Methylmercury*. World Health Organization, Geneva, 1990.

38. Yoshida, M., Watanabe, C., Satoh, H., Kishimoto, T. and Yamamura, Y. : Milk transfer and tissue uptake of mercury in suckling offspring after exposure of lactating maternal guinea pigs to inorganic or methylmercury. *Arch. Toxicol.*, **68**, 174-178, 1994.

Original Article

Factors Affecting Hand Tremor and Postural Sway in Children

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Abstract

Objectives: It is crucial to consider covariates relevant for outcome variables in developing dose-effect relations of environmental hazardous toxins. The aim of this study was to clarify the covariates affecting hand tremor and postural sway in children.

Methods: Hand tremor and postural sway, as well as hair mercury concentrations, were measured in 155 boys and 148 girls at age 7 years.

Results: Current mercury concentrations in child hair ranged from 0.35 to 6.32 µg/g (geometric mean, 1.71 µg/g for boys and 1.58 µg/g for girls), and were not significantly correlated with the neuromotor parameters. All hand tremor and postural sway parameters, except for tremor intensity at 1–6 Hz with non-dominant hand, were significantly larger in the boys than in the girls. Using multiple regression analysis, some postural sway parameters were related negatively to age in the boys and girls (p<0.05), and positively to height (p<0.05). Similarly, hand tremor parameters were positively related to age, height and heart rate either in the boys or in the girls (p<0.05). Also, there were positive relationships between tremor intensity at 1–6 Hz and transversal and sagittal sways at 1–2 Hz and 2–4 Hz (p<0.05).

Conclusions: Heart rate and postural sway, together with age, sex, and height, should be considered in interpreting hand tremor in children. Hand tremor or postural sway may not be so sensitive or specific to methylmercury exposures at levels of less than 7 µg/g in hair.

Key words: hand tremor, postural sway, covariates, methylmercury, child

Introduction

The assessment of neuromotor functions, e.g., postural sway and hand tremor, in children at the developmental stage seems to be troublesome, because various anthropometric factors such as height and body weight interfere with interpretation of the data (1), and children and infants are not little adults and are uniquely vulnerable to environmental factors (2). In addition, few neuromotor tests have been designed to be sensitive enough for the early detection of subtle deficits in involuntary movements (3). For this reason, significant findings have been reported only on the effects of postnatal exposures to hazardous substances such as lead (4, 5), but not on the effects

of prenatal exposures to methylmercury (6), which may have been attributable to the difference between neurotoxic substances. As covariates/confounders involved in neuromotor measures for children, age, height, body weight, head circumference, use of alcohol and marijuana during pregnancy, duration of breastfeeding, socioeconomic status, mother's education, and etc. were considered in previous studies (4, 5, 7–9). Most of them have not been associated with neuromotor functions, or the significance of associations with neuromotor functions has not been described. On the other hand, recent research applying the above neuromotor tests to adults reported that postural sway and heart rate affected hand tremor to some degree (10, 11). For the establishment of neuromotor measures in children, therefore, it is crucial to explore such factors affecting postural sway and hand tremor.

In 7-year-old Japanese children, a cross-sectional study on the neurodevelopmental effects of mercury exposure was conducted (12). In the study, the methylmercury exposure level at parturition was estimated to be between 0.11 and 6.86 (median 1.63) µg/g in hair, but any parameters of postural sway

or hand tremor were not associated with the exposure; at the same time, we could not explain sex difference in the neuromotor parameters. In this case, what sex difference in such parameters resulted from remains unclear. To answer these questions, we tried to scrutinize covariates or confounders affecting the neuromotor function.

Materials and Methods

Subjects

The nature of the procedures used in this study was explained to the parents of the first graders of 28 elementary schools in Akita and Tottori Prefectures, Japan, 14 of which are located near a fishing harbor (12). Children who were born from April 2nd, 1995 to April 1st, 1997 were invited to participate in this study in July–September, 2002 and 2003. In Japan, there were many mines and smelters 30 years ago, and it is probable that soil or water was contaminated by lead, copper or cadmium; therefore, the study population did not include those who came from such areas. Of 327 participating subjects, 24 children were excluded because two children obviously suffered from some neurological disorder (spinal progressive muscular atrophy and epilepsy), one child had a congenital malformation (cleft palate), 20 children had low birth weights (less than 2500 g), and one boy could not perform only the neuromotor test. Thus, 155 boys and 148 girls were enrolled in this study. The study protocol was approved by the ethical review committee at the Akita University School of Medicine.

Methods

Hair samples were collected from the occipital area in all children, by cutting strands of hair close to the scalp. The hair lengths ranged from 1 to 30 (mean 10) cm. Total mercury in aliquots of dried hair samples (15 to 20 mg), rinsed with acetone, was determined by cold vapor atomic absorption spectrophotometry at the National Institute for Minamata Disease (13, 14). A detailed survey of medical records during pregnancy and delivery, including smoking and drinking habits, gestation period, birth weight, and past and present history of illness in the child was conducted, by one medical doctor, at the elementary schools or civic centers where neurodevelopmental examinations were carried out. Also, the medical data were checked by referring to the maternal and child health handbook that mothers had kept for their children.

One trained examiner examined postural sway and hand tremor (11, 12, 15) using the Neurobehavioral Test System (CATSYS 2000, Danish Product Development Ltd., Denmark). Postural sway was measured on a flat floor. The subject was instructed to stand quietly on a platform for 66 s under eyes-open and eyes-closed conditions; Main parameters measured under both conditions were: sway area (mm²: area traveled by the center of force), transversal sway (mm: mean deviation in the medio-lateral direction, Dx) and sagittal sway (mm; mean deviation in the anterior-posterior direction, Dy). The spectral analysis of Dx and Dy was conducted with the fast Fourier transform (FFT) analysis (16) to identify the specific sway; and, square roots (mm) of the power calculated from the power spectrum within the frequency bands of 0–1 Hz, 1–2 Hz, and 2–

4 Hz served as measures of the amount of postural sway for each frequency range. Hand tremor was measured successively for each hand for 16.4 s: the subject was instructed to hold a light stylus as he/she would hold an ordinary pen, with their elbows bent at a right angle and free of body contact or any obstacles. The stylus was held horizontally, parallel to the abdomen at about 10 cm in front of the navel, and the index finger was positioned about 1 cm from the tip of the stylus. The frequency analysis of tremor intensity was conducted with the FFT analysis, and the power spectrum (m/s²) within the frequency bands of 1–6 Hz, 6–10 Hz, and 10–14 Hz, were calculated. Heart rate was measured using the ECG-Amplifier 1271SP (NEC-Sanei Co., Japan) connected to an analog-to-digital converter (sampling time, 1 ms) and a computer, after the subject lay quietly supine for five min (12).

Statistical analysis

The significance of differences in characteristics between boys and girls was analyzed by the Student (or Welch) t test, Mann-Whitney U test or Fisher exact probability. Also, sex differences in neuromotor parameters were tested by the analysis of covariance to adjust for age, height, body weight, heart rate, gestation period, birth weight, mother's smoking and drinking habits during pregnancy, past history of otitis media, and mercury concentration in child's hair. Mother's smoking and drinking habits during pregnancy and past history of otitis media were scored as "absence"=0 and "presence"=1. Multiple regression analysis was employed to evaluate the relations of age, height, body weight, heart rate, and possible covariates to neuromotor parameters in boys and girls, separately. The partial correlation coefficient, as well as the Pearson product-moment correlation coefficient (r), was calculated to determine the relationships between the sway and tremor parameters after controlling for age, height, and heart rate. All analyses, with two-sided p values, were performed using the Statistical Package for the Biosciences (17).

Results

Characteristics of the 155 boys and 148 girls are shown in Table 1. Age, height or body weight did not differ significantly between both sexes. There were significant relationships

Table 1 Characteristics (mean±SD) of Japanese children and their mothers

	155 boys	148 girls	p values
Age (years)	6.9±0.3	6.9±0.3	0.3068
Height (cm)	119.6±5.2	119.8±4.7	0.6777
Body weight (kg)	23.7±4.3	23.6±3.3	0.8488
Heart rate (beats/min)	81.2±9.6	83.6±10.8	0.0409
Body weight at birth (g)	3326±385	3160±344	0.0410
Gestation period (weeks)	39.1±1.3	39.3±1.2	0.4103
Past history of illness in child			
Otitis media	38.7%	38.5%	1.0000[†]
Smoking during pregnancy*	9.7%	4.7%	0.1219[†]
Drinking during pregnancy*	16.1%	9.5%	0.0890[†]

* Mother's habits during pregnancy.
[†] Fisher exact probability.

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between age and height in the boys (r=0.389, p<0.0001) and in the girls (r=0.346, p<0.0001), and significant relationships between height and body weight in the boys (r=0.677, p<0.0001) and in the girls (r=0.624, p<0.0001). The mercury in hair ranged from 0.35 to 5.83 (geometric mean 1.71) μg/g in the boys and from 0.55 to 6.32 (geometric mean 1.58) μg/g in the girls, and the hair mercury did not differ between both sexes (p>0.05). The log-transformed mercury in hair was not significantly correlated with any neuromotor parameters (i.e., postural sway and hand tremor) either in the boys or in the girls (p>0.05 by the Pearson product-moment correlation coefficient).

All neuromotor parameters except for tremor intensity at 1–6 Hz with non-dominant hand were significantly larger in the boys than in the girls (Table 2). In the analysis of covariance, the significant covariates (the correlation and parameter number in parentheses) were age (negative, 15), height (positive, 16), and mother's smoking (positive, 3) and drinking (positive, 8) habits during pregnancy for some postural sway parameters; and, age (positive, 2), height (positive, 2), heart rate (positive, 5), and body weight at birth (positive, 8) for some tremor parameters.

As shown in Table 3, postural sway parameters, in general,

Table 2 Differences in postural sway parameters (mean±SD) between Japanese 155 boys and 148 girls after adjusting for ten possible covariates such as age, height, and body weight: results of analysis of covariance

	155 boys	148 girls	p value
Postural sway:			
Eyes open			
Sway area, mm²	865±550	563±293	<0.0001
Transversal sway (Dx), mm	5.46±1.84	4.45±1.22	<0.0001
Dx at 0–1 Hz, mm	6.82±2.32	5.55±1.49	<0.0001
Dx at 1–2 Hz mm	0.90±0.33	0.75±0.24	<0.0001
Dx at 2–4 Hz, mm	0.39±0.15	0.32±0.10	<0.0001
Sagittal sway (Dy), mm	5.63±1.84	4.80±1.54	<0.0001
Dy at 0–1 Hz, mm	7.04±2.38	5.98±1.88	<0.0001
Dy at 1–2 Hz, mm	1.11±0.47	0.90±0.32	<0.0001
Dy at 2–4 Hz, mm	0.50±0.19	0.41±0.15	<0.0001
Eyes closed			
Sway area (mm²)	1263±876	808±488	<0.0001
Transversal sway (Dx), mm	6.09±1.90	4.92±1.39	<0.0001
Dx at 0–1 Hz, mm	7.60±2.33	6.15±1.70	<0.0001
Dx at 1–2 Hz, mm	1.26±0.64	0.99±0.34	<0.0001
Dx at 2–4 Hz, mm	0.55±0.26	0.44±0.17	<0.0001
Sagittal sway (Dy), mm	6.23±1.76	5.20±1.43	<0.0001
Dy at 0–1 Hz, mm	7.71±2.20	6.46±1.84	<0.0001
Dy at 1–2 Hz, mm	1.62±0.62	1.32±0.46	<0.0001
Dy at 2–4 Hz, mm	0.71±0.31	0.57±0.22	<0.0001
Tremor intensity, m/s²:			
Dominant hand			
Total	0.192±0.071	0.169±0.053	0.0034
1–6 Hz	0.144±0.060	0.127±0.044	0.0078
6–10 Hz	0.108±0.036	0.098±0.032	0.0126
10–14 Hz	0.050±0.019	0.044±0.016	0.0054
Non-dominant hand			
Total	0.219±0.079	0.201±0.069	0.0387
1–6 Hz	0.168±0.064	0.158±0.058	0.1705
6–10 Hz	0.119±0.046	0.107±0.038	0.0182
10–14 Hz	0.057±0.026	0.051±0.021	0.0215

were related negatively to age and positively to height. Body weight was positively related to the total Dy and the Dy at 0–1 Hz with eyes open in the boys, but negatively to the Dx at 1–2 Hz and Dx at 2–4 Hz with eyes open in the girls. In including mother's smoking and drinking habits during pregnancy in independent variables of the multiple regression analysis, drinking habit, in addition to age and height, was positively related to twelve sway parameters only in the boys (p<0.05, data not shown).

The total tremor intensity and component tremor intensities at 6–10 Hz and 10–14 Hz for the dominant hand were positively related to age in the girls, and most of tremor parameters were positively related to height in the boys (Table 4); also, heart rate was positively related to most of tremor parameters in the boys. In adding birth weight and mother's smoking and drinking habits to the independent variables, birth weight, together with age, height and heart rate, was positively related to eight tremor parameters only in the boys (p<0.05, data not shown).

With respect to the association between the tremor and sway parameters (Table 5), tremor intensity at 1–6 Hz was significantly correlated with the Dx at 1–2 Hz and 2–4 Hz and Dy at 2–4 Hz with eyes open among the boys and girls. Among the girls, tremor intensity at 1–6 Hz was also significantly correlated with some sway parameters with eyes closed.

Discussion

In this study, the children with hair mercury levels of 0.35–6.32 μg/g showed no significant relations of current mercury exposure to either postural sway or hand tremor parameters. When maternal hair mercury concentration was used as a proxy for mercury exposure at parturition, we failed to find any significant relationships between prenatal methylmercury exposures and neuromotor variables in the same children (12). Similarly, a dose-effect association of methylmercury with postural sway parameters has not been observed in the Faroese birth cohort (6). Methylmercury exposures in both populations resulted mainly from seafood diet (18, 19). On the other hand, postural instability and increased hand tremor have been reported in adults exposed to lead, manganese, arsenic, and organic solvents (11, 20–23). Therefore, it is suggested that postural sway or hand tremor may not be so sensitive or specific to prenatal low-level exposures to methylmercury, at least less than 7 μg/g in hair.

The present study of 7-year-old children showed that postural sway and hand tremor differed between both sexes. In the Faroese birth cohort, difference in postural balance observed between both sexes at age 7 years (24). Also, boys under 10 years swayed more than girls (7, 9). Thus, this difference at the developmental stage may have originated not from anthropometric factors, but from certain biological factors such as sex chromosome or from exposures to a certain neurotoxin other than methylmercury. In addition, Darlington et al. (25) reported that although the menstrual cycle phase had no significant effect on sagittal sway, it did significantly affect transversal sway. In healthy elderly people, however, gender-based balance differences were not shown during quiet standing

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Table 3 Relations of age, height, body weight, and heart rate to postural sway parameters in Japanese children: Results of multiple regression analysis

	Standard regression coefficient				Multiple correlation coefficient
	Age	Height	Body weight	Heart rate	
155 boys:					
Eyes open					
Sway area	-0.200*	0.310**	0.024	-0.041	0.318**
Transversal sway (Dx)	-0.176*	0.343**	-0.068	-0.084	0.301**
Dx at 0–1 Hz	-0.191*	0.349**	-0.066	-0.085	0.309**
Dx at 1–2 Hz	-0.094	0.293*	-0.064	-0.091	0.261*
Dx at 2–4 Hz	-0.154	0.288*	-0.065	0.003	0.234
Sagittal sway (Dy)	-0.071	0.074	0.258*	-0.022	0.306**
Dy at 0–1 Hz	-0.089	0.077	0.248*	-0.016	0.298**
Dy at 1–2 Hz	-0.131	0.246*	-0.048	0.048	0.205
Dy at 2–4 Hz	-0.172*	0.319**	-0.095	0.083	0.259*
Eyes closed					
Sway area	-0.194*	0.279*	0.016	-0.088	0.299**
Transversal sway (Dx)	-0.166	0.362**	-0.081	-0.046	0.298**
Dx at 0–1 Hz	-0.169	0.363**	-0.070	-0.046	0.304**
Dx at 1–2 Hz	-0.156	0.212	-0.046	-0.038	0.192
Dx at 2–4 Hz	-0.139	0.280*	-0.122	-0.018	0.208
Sagittal sway (Dy)	-0.063	0.090	0.154	-0.101	0.249*
Dy at 0–1 Hz	-0.073	0.077	0.163	-0.106	0.249*
Dy at 1–2 Hz	-0.169	0.204	-0.042	0.000	0.190
Dy at 2–4 Hz	-0.137	0.228	-0.113	0.046	0.179
148 girls:					
Eyes open					
Sway area	-0.180*	0.234*	-0.069	-0.039	0.219
Transversal sway (Dx)	-0.189*	0.248*	-0.145	-0.082	0.233
Dx at 0–1 Hz	-0.195*	0.248*	-0.145	-0.096	0.241
Dx at 1–2 Hz	-0.159	0.378***	-0.221*	0.033	0.281*
Dx at 2–4 Hz	-0.146	0.322**	-0.219*	-0.005	0.243
Sagittal sway (Dy)	-0.099	0.149	0.097	0.013	0.216
Dy at 0–1 Hz	-0.087	0.157	0.086	0.021	0.211
Dy at 1–2 Hz	-0.174	0.070	0.023	-0.033	0.173
Dy at 2–4 Hz	-0.181*	0.076	-0.036	-0.033	0.170
Eyes closed					
Sway area	-0.263**	0.214	-0.098	-0.057	0.262*
Transversal sway (Dx)	-0.295***	0.271*	-0.102	-0.098	0.314**
Dx at 0–1 Hz	-0.294***	0.263*	-0.088	-0.104	0.314**
Dx at 1–2 Hz	-0.286**	0.283*	-0.172	0.062	0.298**
Dx at 2–4 Hz	-0.218*	0.201	-0.144	0.037	0.224
Sagittal sway (Dy)	-0.221*	0.228*	-0.014	-0.041	0.257*
Dy at 0–1 Hz	-0.219*	0.246*	-0.034	-0.040	0.258*
Dy at 1–2 Hz	-0.285**	0.139	-0.017	0.000	0.267*
Dy at 2–4 Hz	-0.211*	0.184	-0.105	0.050	0.214

* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

(26). In either case, it is important to consider gender-specific neuromotor dysfunctions due to environmental or occupational hazardous substances.

In this study, age was negatively associated with postural sway parameters in both boys and girls. Postural sway decreased linearly with age from 2 to 14 years (8); also, sway amplitude in 64 healthy children aged between 3.5 and 17 years decreased with age for boys, but not for girls (7). In another research, Usui et al. (9) reported that upright postural sway decreased markedly between the ages of 3 to 5 years and then slowly after six years of age. By contrast, postural sway in adults is known to increase with advancing age (27, 28). Thus,

postural sway appears to decrease with increasing age in children at ages of less than 15 years, although postural stability in adults is worse in elderly people. Nevertheless, it may be difficult to identify the turning-point of aging effects on postural sway, because there seems to be no single anthropometric factor that explains the variations in body-balancing movements during standing (1).

Concerning the hand tremor, age was positively related to two tremor parameters only for the dominant hand of girls (Table 4); while, the age-range interval of our study was within 12 months. On the other hand, the amplitude of finger tremor has been reported to be significantly higher in 22 children at

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Table 4 Relations of age, height, body weight, and heart rate to tremor intensity in Japanese children: Results of multiple regression analysis

	Standard regression coefficient				Multiple correlation coefficient
	Age	Height	Body weight	Heart rate	
155 boys:					
Dominant hand					
Total	−0.011	0.249*	−0.091	0.179*	0.240
1–6 Hz	−0.063	0.288*	−0.052	0.165*	0.267*
6–10 Hz	0.079	0.167	−0.146	0.232**	0.262*
10–14 Hz	0.018	0.192	−0.154	0.157	0.201
Non-dominant hand					
Total	−0.151	0.350**	−0.067	0.188*	0.323**
1–6 Hz	−0.124	0.360**	−0.049	0.183*	0.331**
6–10 Hz	−0.154	0.298*	−0.130	0.198*	0.289*
10–14 Hz	−0.168	0.263*	−0.099	0.153	0.255*
148 girls:					
Dominant hand					
Total	0.213*	−0.078	−0.010	−0.003	0.202
1–6 Hz	0.141	−0.029	0.027	−0.055	0.148
6–10 Hz	0.290**	−0.106	−0.052	0.042	0.288*
10–14 Hz	0.267**	−0.134	−0.080	0.041	0.288*
Non-dominant hand					
Total	0.064	0.080	−0.137	0.076	0.156
1–6 Hz	0.065	0.075	−0.129	0.052	0.139
6–10 Hz	0.067	0.037	−0.101	0.142	0.184
10–14 Hz	0.028	0.080	−0.131	0.103	0.156

$* p<0.05$, $** p<0.01$.

Table 5 Partial correlation coefficients[a] between tremor intensity and postural sway parameters among Japanese children

	Tremor intensity (1–6 Hz)			
	155 boys		148 girls	
	Dominant hand	Non-dominant hand	Dominant hand	Non-dominant hand
Eyes open				
Sway area	0.027	0.008	0.197*	0.129
Transversal sway (Dx)	0.036	0.024	0.091	−0.021
Dx at 0–1 Hz	0.028	0.007	0.102	−0.014
Dx at 1–2 Hz	0.217**	0.166*	0.234**	0.249**
Dx at 2–4 Hz	0.226**	0.174*	0.314***	0.295***
Sagittal sway (Dy)	0.005	−0.070	0.114	0.076
Dy at 0–1 Hz	−0.014	−0.082	0.109	0.083
Dy at 1–2 Hz	0.121	0.107	0.197*	0.234**
Dy at 2–4 Hz	0.220**	0.177*	0.270**	0.278***
Eyes closed				
Sway area	0.026	0.018	0.274***	0.220**
Transversal sway (Dx)	0.039	0.031	0.123	0.067
Dx at 0–1 Hz	0.044	0.029	0.144	0.081
Dx at 1–2 Hz	0.068	0.075	0.154	0.170*
Dx at 2–4 Hz	0.156	0.115	0.224**	0.212*
Sagittal sway (Dy)	0.100	0.086	0.156	0.204*
Dy at 0–1 Hz	0.068	0.053	0.177*	0.219**
Dy at 1–2 Hz	0.107	0.091	0.219**	0.220**
Dy at 2–4 Hz	0.179*	0.153	0.224**	0.238**

[a] Age, height and heart rate were used as confounders in all analyses.
$* p<0.05$, $** p<0.01$, $*** p<0.001$.

ages 7–9 years than in 22 children at ages 13–15 years (29), which included boys and girls. Also, tremor acceleration in subjects at ages 10–90 years has been reported to increase with advancing age, although the author did not seem to consider sex difference (30). From these reports, hand tremor parameters appear to depend on age, but the relation remains uncertain in children aged between 7 and 15 years.

In the present study, height was positively associated with

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postural sway and hand tremor parameters. Usui et al. (9) described that the sway of the center of gravity was larger in tall than in short people, and correction for height was made. On the contrary, some research showed that height and age were negatively associated with sway variables (4, 7). In children at the developmental stage (i.e., aged between 4 and 10 years), the relationship between age and height seems to be considerably strong; for example, the correlation coefficient was 0.96 for 76 children aged between 2 and 14 years (8). For this reason, the relation of height to neuromotor parameters may have been affected by the strong collinearity between age and height in the multivariate analysis. Nevertheless, postural sway would not be independent of height of the center of gravity.

Body weight was related positively to the sagittal sway in the boys of this study, and negatively to the transversal sway in the girls. Also, body weight had a very close relation to height in the boys and girls. Likewise, Riach and Hayes (8) reported that the correlation coefficient between body weight and either age or height was 0.90 or 0.93, respectively. In the latter case, the effect of body weight may be changeable due to the collinearity between height and body weight. Hence, Bhattacharya et al. (4), attempting to clarify the effect of early lead exposure on children's postural balance, used body weight and other possible confounders, together with age, sex, and height, as independent variables of multiple regression analysis. On the basis of these findings, it is speculated that if the study population did not include children with excessive obesity or emaciation, body weight would hardly affect postural sway by controlling for age and height. Rather, too many covariates should not be used to avoid overmatching in an intermediate between exposure and disease (31), and the estimation accompanying such overmatching may be biased toward the null hypothesis (32).

In the present study, heart rate was positively related to tremor intensity at 1–6 Hz and 6–10 Hz in the boys. A similar finding has been observed in female adults (10). Originally, inapparent minor tremors on the human body surface were described in 1930s (33, 34), and Ozaki et al. (35) reported that heart beat, in addition to muscle tonus, might play an important role in minor tremors under various physiological conditions.

Likewise, Gallasch and Kenner (36) proved that the R peak on the electrocardiogram triggered microvibrations in the supported arm. In our study, since the subjects were instructed to hold a light stylus, free of body contact or any obstacles, the effect of heart rate on hand tremor may have readily been detectable. In this sense, heart rate should be checked for subjects with palpitation.

Postural sway parameters in the boys and girls of our study were significantly linked with tremor intensity at 1–6 Hz (Table 5). This finding is concordant with two previous reports (3, 11). It is likely that involuntary postural sway at 1–4 Hz intervened directly in finger tremor via the humerus and forearm, because the frequency of tremor was almost similar to that of postural sway.

In conclusion, age, sex, and height are suggested to be the major developmental variables contributing to neuromotor measures for children. Heart rate and postural sway can also affect hand tremor not only in adults but also in children. In addition, body weight at birth and mother's drinking habit during pregnancy were suggested to be associated with the neuromotor function in Japanese boys, but the implication of these two factors awaits further research. The most important thing is that these neuromotor tests in a series of study should be carried out carefully by one trained examiner, although such tests seem to be apparently simple and feasible for examiners. Letz (37) stresses the importance of motivational influences that can affect the reliability and the validity of the tests, and two or more examiners may produce a measurement bias (38). In fact, there are only a few studies that have provided statistically significant findings obtained using the CATSYS system (5, 11, 22, 23, 39, 40, 41).

Acknowledgments

References

(1) Kejonen P, Kauranen K, Vanharanta H. The relationship between anthropometric factors and body-balancing movements in postural balance. Arch Phys Med Rehabil. 2003; 84:17–22.

(2) Landrigan PJ, Kimmel CA, Correa A, Eskenazi B. Children's health and the environment: public health issues and challenges for risk assessment. Environ Health Perspect. 2003; 112:257–265.

(3) Després C, Richer F, Roberge M-C, Lamoureux D, Beuter A. Standardization of quantitative tests for preclinical detection of neuromotor dysfunctions in pediatric neurotoxicology. Neurotoxicology. 2005;26:385–395.

(4) Bhattacharya A, Shukla R, Dietrich K, Bornshein R, Berger O. Effect of early lead exposure on children's postural balance. Dev Med Child Neurol. 1995;37:861–878.

(5) Després C, Beuter A, Richer F, Poitras K, Veilleux A, Ayotte P, Dewailly E, Saint-Amour D, Muckle G. Neuromotor functions in Inuit preschool children exposed to Pb, PCBs, and Hg. Neurotoxicol Teratol. 2005;27:245–257.

(6) Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, Murata K, Sørensen N, Dahl R, Jørgensen PJ. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol Teratol. 1997;19:417–428.

(7) Odenrick P, Sandstedt P. Development of postural sway in the normal child. Hum Neurobiol. 1984;3:241–244.

(8) Riach CL, Hayes KC. Maturation of postural sway in young children. Dev Med Child Neurol. 1987;29:650–658.

(9) Usui N, Maekawa K, Hirasawa Y. Development of the upright postural sway of children. Dev Med Child Neurol. 1995;37:

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985–996.

(10) Ishii N, Iwata T, Dakeishi M, Murata K. Effects of shift work on autonomic and neuromotor functions in female nurses. J Occup Health. 2004;46:352–358.

(11) Iwata T, Mori H, Dakeishi M, Onozaki I, Murata K. Effects of mixed organic solvents on neuromotor functions among workers in Buddhist altar manufacturing factories. J Occup Health. 2005;47:143–148.

(12) Murata K, Sakamoto M, Nakai K, Weihe P, Dakeishi M, Iwata T, Liu X-J, Ohno T, Kurosawa T, Satoh H. Effects of methylmercury on neurodevelopment in Japanese children in relation to the Madeiran study. Int Arch Occup Environ Health. 2004;77:571–579.

(13) Akagi H, Nishimura H. Speciation of mercury in the environment. In: Suzuki T, Imura N, Clarkson TW editors. Advances in Mercury Toxicology. New York: Plenum Press; 1991. P. 53–76.

(14) Sakamoto M, Kubota M, Liu XJ, Murata K, Nakai K, Satoh H. Maternal and fetal mercury and n-3 polyunsaturated fatty acids as a risk and benefit of fish consumption to fetus. Environ Sci Technol. 2004;38:3860–3863.

(15) Després C, Lamoureux D, Beuter A. Standardization of a neuromotor test battery: the CATSYS system. Neurotoxicology. 2000;21:725–735.

(16) Cooley JW, Tukey JW. An algorithm for the machine calculation of complex Fourier series. Math Comp. 1965; 19:297–301.

(17) Murata K, Yano E. Medical Statistics for Evidence-Based Medicine with SPBS User's Guide. Tokyo: Nankodo Publisher; 2002.

(18) 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;47:185–195.

(19) Iwasaki Y, Sakamoto M, Nakai K, Oka T, Dakeishi M, Iwata T, Satoh H, Murata K. Estimation of daily mercury intake from seafood in Japanese women: Akita cross-sectional study. Tohoku J Exp Med. 2003;200:67–73.

(20) Yokoyama K, Araki S, Murata K, Nishikitani M, Nakaaki K, Yokota J, Ito A, Sakata E. Postural sway frequency analysis in workers exposed to n-hexane, xylene, and toluene: assessment of subclinical cerebellar dysfunction. Environ Res. 1997;74:110–115.

(21) Yokoyama K, Araki S, Murata K, Morita Y, Katsuno N, Tanigawa T, Mori N, Yokota J, Ito A, Sakata E. Subclinical vestibule-cerebellar, anterior cerebellar lobe and spinocerebellar effects in lead workers in relation to concurrent and past exposure. Neurotoxicology. 1997;18:371–380.

(22) Gerr F, Letz R, Ryan PB, Green RC. Neurological effects of environmental exposure to arsenic in dust and soil among humans. Neurotoxicology. 2000;21:475–488.

(23) Bast-Pettersen R, Ellingsen DG, Hetland SM, Thomassen Y. Neuropsychological function in manganese alloy plant workers. Int Arch Occup Environ Health. 2004;77:277–287.

(24) Araki S, Murata K, Yokoyama K, Okajima F, Grandjean P, Weihe P. Neuroelectrophysiological study of children in low-level methylmercury exposure in Faroe Islands: methodology and preliminary findings. In: Proceedings of the International Symposium on Assessment of Environmental Pollution and Health Effects from Methylmercury. Minamata: National Institute for Minamata Disease; 1994. P. 141–151.

(25) Darlington CL, Ross A, King J, Smith PF. Menstrual cycle effects on postural stability but not optokinetic function. Neurosci Lett. 2001;307:147–150.

(26) Wolfson L, Whipple R, Derby CA, Amerman P, Nashner L. Gender differences in the balance of healthy elderly as demonstrated by dynamic posturography. J Gerontol. 1994; 49:M160–167.

(27) Du Pasquier RA, Blanc Y, Sinnreich M, Landis T, Burkhard P, Vingerhoets FJ. The effect of aging on postural stability: a cross sectional and longitudinal study. Neurophysiol Clin. 2003;33:213–218.

(28) Fujita T, Nakamura S, Ohue M, Fujii Y, Miyauchi A, Takagi Y, Tsugeno H. Effect of age on body sway assessed by computerized posturography. J Bone Miner Metab. 2005;23: 152–156.

(29) Birmingham AT, Wharrad HJ, Williams EJ. The variation of finger tremor with age in man. J Neurol Neurosurg Psychiatry. 1985;48:788–798.

(30) Lakie M. Is essential tremor physiological? In: Findley LJ, Koller WC editors. Handbook of Tremor Disorders. New York: Marcel Dekker, Inc; 1995. P. 165–183.

(31) Rothman KJ, Greenland S. Modern Epidemiology, 2nd edn. Hagerstown: Lippincott-Raven Publisher; 1998.

(32) Budtz-Jørgensen E, Grandjean P, Jørgensen PJ, Weihe P, Keiding N. Association between mercury concentrations in blood and hair in methylmercury-exposed subjects at different ages. Environ Res. 2004;95:385–393.

(33) Travis LE, Hunter TA. Tremor frequencies. J Gen Psychol. 1931;5:255–260.

(34) Jasper HH, Andrews HL. Brain potential and voluntary muscle activity in man. J Neruophysiol. 1938;1:87–100.

(35) Ozaki T, Sato K, Awazu T, Mimura K, Honda N, Teramoto S, Kitajima K. Some observations on minor tremors related to heart beat. Jpn J Physiol. 1962;12:484–493.

(36) Gallasch E, Kenner T. Characterisation of arm microvibration recorded on an accelerometer. Eur J Appl Physiol. 1997;75: 226–232.

(37) Letz R. Continuing challenges for computer-based neuropsychological tests. Neurotoxicology. 2003;24:31–39.

(38) Grandjean P, White RF, Weihe P. Evidence of developmental methylmercury toxicity. In: Abstract of 6th International Conference on Mercury as a Global Pollutant. Minamata: National Institute for Minamata Disease; 2001. P. 233.

(39) Netterstrøm B, Guldager B, Heebøll J. Acute mercury intoxication examined with coordination ability and tremor. Neurotoxicol Teratol. 1996;18:505–509.

(40) Frumkin H, Letz R, Williams PL, Gerr F, Pierce M, Sanders A, Elon L, Manning CC, Woods JS, Hertzberg VS, Mueller P, Taylor BB. Health effects of long-term mercury exposure among chloralkali plant workers. Am J Ind Med. 2001;39:1–18.

(41) Iwata T, Yano E, Karita K, Dakeishi M, Murata K. Critical dose of lead affecting postural balance in workers. Am J Ind Med. 2005;48:319–325.

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ORIGINAL ARTICLE

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Subclinical effects of prenatal methylmercury exposure on cardiac autonomic function in Japanese children

Abstract *Objectives*: The subclinical effects of prenatal exposure to methylmercury from fish consumption on the cardiac autonomic function were assessed in 136 Japanese 7-year-old children recruited for this study. *Methods*: Samples of child's hair and dry umbilical cord preserved were collected, and hair mercury and cord tissue methylmercury concentrations were determined as current and prenatal exposure biomarkers, respectively. Cardiac autonomic indicators of parasympathetic and sympathetic activities were calculated from the electrocardiographic RR intervals measured. *Results*: In the children, the cord tissue methylmercury (0.017–0.367, median 0.089 μg/g) was not significantly correlated with the hair mercury (0.43–6.32, median 1.66 μg/g). The cord tissue methylmercury was related negatively to parasympathetic components of cardiac autonomic indicators ($P < 0.05$) and positively to sympathovagal indices ($P < 0.05$), even after correction for possible confounders such as age and sex, although the hair mercury was not significantly correlated with any cardiac autonomic indicators. *Conclusions*: Despite the potential limitations involved in the retrospective study, these findings suggest that prenatal methylmercury exposure (median of estimated maternal hair mercury at parturition, 2.24 μg/g) may be associated with reduced parasympathetic activity and/or sympathovagal shift.

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Introduction

Since 1956, methylmercury pollution has been found near Minamata Bay in Japan, and the pollution, which was discharged from a chemical factory, was so widespread that the number of victims increased to more than 2,000. Also, all children identified as suffering from the most severe form of congenital Minamata disease (i.e., methylmercury poisoning) expressed mental retardation, primitive reflexes, cerebellar ataxia, disturbances in physical growth, dysarthria, and limb deformities (Harada 1995). The diagnosis of Minamata disease was easy in typical and severe cases, but was difficult in "mild" cases ranging from partly damaged to healthy (Igata 1993). On the other hand, recent interests of investigators seem to be the subclinical, but not clinical, effects of low-level exposures to methylmercury on child neurodevelopment. Therefore, it is crucial to discover more sensitive and reliable outcome variables, together with specific exposure biomarkers.

Concerning the neurodevelopmental effects of prenatal methylmercury exposure, the Faroese birth cohort study and Seychelles child development study have provided different conclusions and several uncertainties remain because of issues involved in exposure biomarkers, neurodevelopmental endpoints, and confounders (National Research Council 2000). The implications, judging from these two comparative studies, are that neuropsychological measures may depend on social and cultural factors including race and language, and that a key to resolving whether exposure has harmed the fetus may lie in neurophysiological measures such as brainstem auditory-evoked potentials and electrocardiographic (ECG) RR interval variability (Murata and Dakeishi 2005). In addition, the most serious problem may be the extreme scarcity of data from any place other than the Faroe Islands

and Seychelles (Davidson et al. 1998; Grandjean et al. 1997, 2004; Myers et al. 2003; Sørensen et al. 1999). Since each country has a unique food culture, further research in the third area should be done to draw a conclusion on risk assessment of methylmercury (Clarkson 2002).

Short-term variations in heart rate are seen at all ages and are an important sign of normal homeostatic mechanisms of the cardiovascular system (Finley and Nugen 1995). Especially, beat-to-beat heart rate variability (HRV) with frequency-domain analysis has emerged as a noninvasive method to assess cardiac autonomic activity quantitatively. Also, HRV has been reported to be highly reproducible under standardized conditions (Batten et al. 2000; Massin and von Bernuth 1997; Silvetti et al. 2001). By using the HRV, it has been suggested that subjects with low HRV had an adverse cardiovascular risk profile and an elevated risk of incident coronary heart disease (CHD) and death (Dekker et al. 2000), or that prenatal methylmercury exposure was associated with low HRV (Grandjean et al. 2004). In this way, HRV is considered to be a promising tool to provide a marker of less favorable health (Murata and Araki 1996), and will be much applicable to risk assessment on child neurodevelopment.

We conducted a cross-sectional study on the developmental effects of methylmercury exposure in Japanese 7-year-old children (Murata et al. 2004). According to the food frequency questionnaire (FFQ) done at the time of the examination, fish that their mothers had eaten was sardine, flatfish, mackerel, codfish, skipjack tuna, salmon, eel, swordfish, tuna, etc.; and, a significant relationship between the estimated daily mercury intake from seafood and current hair mercury concentration was observed in the mothers ($r = 0.335$), indicating that main methylmercury exposures in Japanese women resulted from fish consumption (Iwasaki et al. 2003). In the study, though we used maternal hair mercury concentration at that time as a proxy for mercury exposure at birth, it might not be the best biomarker of prenatal exposure. Also, it has been suggested that a myth that hair mercury concentration reflects the methylmercury concentration circulating in the blood may collapse if a study population includes subjects with artificial hair-waving (Dakeishi et al. 2005). The mercury level in the umbilical cord tissue has been reported to be associated well with that in cord blood (Dalgård et al. 1994), and most Japanese families treasure a small piece of the umbilical cord of the child as a birth memento (Akagi et al. 1998). For this reason, we tried to collect dry umbilical cord samples of the children from parents who participated in our study. Then, a retrospective study with the methylmercury concentration in the cord tissue was carried out to assess the subclinical effects of prenatal exposure on cardiac autonomic function, as well as the reaction time that was used in both the Faroes and Seychelles studies (Grandjean et al. 1997; Myers et al. 2003).

Materials and methods

Subjects

The nature of the procedures used in this study was explained to parents with a first grader, of 28 elementary schools in Akita and Tottori Prefectures, Japan, 14 of which were located near the fishing harbor. In accordance with the preceding studies on the risk assessment of methylmercury (Grandjean et al. 1997; Murata et al. 1999a), mothers and their children, who were born in the period of April 2nd 1995 to April 1st 1997, were invited for this study during the period of July–September in 2002 and 2003. In Japan, there were many mines and smelters 30 years ago, and it was probable that soil or water has been contaminated by lead, copper or cadmium; therefore, the study population did not include those who came from such areas. Of 327 participating subjects (Murata et al. 2004), 145 provided a piece of dry umbilical cord sample for our proposal. In the light of the previous exclusion criteria (Murata et al. 2004), two children with neurological disorders (spinal progressive muscular atrophy and epilepsy), one with a congenital malformation (cleft palate), and six with low birth weight (less than 2,500 g) were counted out. In total, 136 children were enrolled as subjects of this research. The study protocol was approved by the ethical review committee at the Akita University School of Medicine.

Methods

Hair samples were collected, by cutting strands of hair close to the scalp, from the occipital area in all children. The hair length ranged from 1 to 30 cm (mean 10 cm). Total mercury in aliquots of dried hair samples (15–20 mg), rinsed with acetone, was determined by the cold vapor atomic absorption spectrophotometry method at the National Institute for Minamata Disease (Akagi et al. 2000; Sakamoto et al. 2004). The method involved sample digestion with HNO_3, $HClO_4$, and H_2SO_4 followed by reduction to elemental mercury vapor by $SnCl_2$. The detection limit was 0.01 ng/g. Methylmercury in each umbilical cord sample, after crushing it and removing blood cells from it with tweezers, was determined by gas chromatography with electron capture detector (GC-ECD) according to the method of Akagi et al. (2000), in order to avoid contamination due to inorganic mercury compounds (e.g., mercurochrome of disinfectant). The method involved sample digestion with KOH–ethanol and subsequently, under a slightly acidic condition, the fatty content was removed using n-hexane. After extraction with dithizone–toluene, methylmercury was back-extracted with a slightly alkaline sodium sulfide solution. The excess sulfide ions were then removed as hydrogen sulfide by purging with nitrogen gas after slight acidification with HCl solution. Methylmercury was then re-extracted with a small por-

tion of dithizone–toluene. The extract was washed with NaOH solution to remove the excess dithizone, and was then slightly acidified with HCl and analyzed using GC-ECD. The detection limit was 0.01 ng/g. The precision and accuracy of these methods were repeatedly verified by inter-laboratory calibration exercises including the analysis of standard reference material such as IAEA-085, 086 and 142. The methylmercury in the cord tissue and total mercury in hair were used as the prenatal and current exposure biomarkers, respectively.

A detailed survey of medical records during pregnancy and delivery, including smoking/drinking habit, gestation period and birth weight, past/present history of illness in the child, and dietary habits in the mother, was conducted by one medical doctor at the elementary schools or civic centers where neurodevelopmental examinations were done. Also, the medical data were checked by reference to the maternal and child health booklet that she had kept for her child and in which she and medical doctors recorded during pregnancy and at the time of parturition, health examination or medical observation. At that time, height and body weight in the child were measured to compute body mass index (BMI, kg/m^2).

The HRV was measured by a trained examiner, using the ECG-Amplifier 1271SP (NEC-Sanei Co., Japan) connected to an analog-to-digital converter (sampling time, 1 ms) and a computer. After the subject remained quietly in the supine position at least for 5 min, 300 RR intervals were measured, and consecutive 100 RR intervals with the minimal standard deviation were automatically extracted to avoid measurement error (Grandjean et al. 2004; Murata and Araki 1996; Murata et al. 1992). The power spectrum of RR intervals was computed by autoregressive spectral analysis. The spectrum of each of two components, i.e., the high-frequency (HF) component at the center frequency of 0.15–0.4 Hz and low-frequency (LF) component at 0.01–0.15 Hz, was separated by component analysis (Murata et al. 2004). Each component coefficient of variation (i.e., CCV$_{HF}$ and CCV$_{LF}$, %) was defined as the ratio of the square root of each component power spectral density (PSD$_{HF}$ and PSD$_{LF}$, ms^2) to the mean value of RR intervals (ms). The CCV$_{HF}$ and PSD$_{HF}$ reflect the vagal activity (Murata and Araki 1996; Pagani et al. 1986; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996), and the CCV$_{LF}$ and PSD$_{LF}$ are thought to represent the sympathetic activity. Also, the LF/HF ratio and %LF are calculated from PSD$_{LF}$/PSD$_{HF}$ and 100 × PSD$_{LF}$/(PSD$_{LF}$ + PSD$_{HF}$), respectively. The heart rate-corrected QT interval (QTc) was automatically calculated by the ECG-9202 electrocardiograph (Nihon Kohden Co., Japan), from the RR and QT intervals on ECG according to Bazett's formula (Murata et al. 1999b). Reaction time to a sound stimulus was measured with right and left hands, using the Neurobehavioral Test System (CATSYS 2000, Danish Product Development Ltd, Denmark) (Després et al. 2000; Murata et al. 2004).

Data analyses

Logarithmic transformation of methylmercury and mercury concentrations was used because of skewed distributions. Likewise, outcome variables excluding %LF, RR and QTc intervals, and reaction time, required logarithmic transformation to obtain a better fit of the regression models. The correlation coefficients between exposure biomarkers and outcome variables were calculated; also, the relations of two exposure levels (i.e., methylmercury in the cord tissue and mercury in hair) to each endpoint were tested by multiple regression analysis to adjust for age, sex, gestation period, and smoking and drinking status during pregnancy (and, plus BMI). Smoking (or drinking) status was scored as "nonsmoker or ex-smoker (or nondrinker)" = 0 and "smoker (or drinker)" = 1. All analyses, with two-sided P values, were performed using the Statistical Package for the Biosciences (Murata and Yano 2002).

Results

Table 1 represents the characteristics at birth and at the time of examination in Japanese children with and without umbilical cord. In the 136 children with umbilical cord, the methylmercury level in the cord tissue ranged from 0.017 to 0.367 µg/g (median 0.089), and the current mercury level in hair was between 0.43 and 6.32 µg/g (median 1.66). The methylmercury in the cord tissue was not significantly correlated with the mercury in hair ($r = -0.107$, $P = 0.217$). Among 136 mothers of the children with umbilical cord, 15 mothers reported drinking a little bit of alcohol during pregnancy, but no mothers ate shark or whale. In the children with umbilical cord, there were seen to be no abnormal ECG findings. The cardiac autonomic indicators or reaction time did not differ significantly between the 75 boys and 61 girls (Table 2).

Among the 136 children with umbilical cord, the methylmercury in the cord tissue was correlated negatively with the CCV$_{HF}$ (Fig. 1) and PSD$_{HF}$ ($r = -0.190$, $P = 0.027$), and positively with the LF/HF ratio (Fig. 1) and %LF ($r = 0.207$, $P = 0.016$), using the Pearson product–moment correlation coefficient; however, the remaining HRV parameters and reaction time were not significantly correlated with either the methylmercury in the cord tissue or mercury in hair ($P > 0.05$). Also, the methylmercury in the cord tissue was significantly related to the CCV$_{HF}$ ($P = 0.022$), PSD$_{HF}$ ($P = 0.023$), LF/HF ratio ($P = 0.017$), and %LF ($P = 0.017$) when controlling for possible confounders (Table 3). These results were similar in adding BMI in independent variables of Table 3.

Table 1 Characteristics at birth and at the time of examination in Japanese children at age 7 years

	136 children with umbilical cord			182 children without umbilical cord		
	Mean (or, Number, %)	SD	5 and 95 percentiles	Mean (or, Number, %)	SD	5 and 95 percentiles
Records during pregnancy and delivery						
Body weight at birth (g)	3,203	359	2,656–3,886	3,133	460	2,420–3,811
Gestation period (weeks)	39.1	1.3	37–41	39.1	1.4	37–41
Natural delivery	120 (88.2 %)			163 (89.6 %)		
Mother's smoking during pregnancy	10 (7.4 %)			14 (7.7 %)		
Mother's drinking during pregnancy	15 (11.0 %)			26 (14.3 %)		
Past history–febrile convulsion	7(5.1 %)			22 (12.1 %)		
Data at the time of examination						
Age (years)	6.9	0.3	6.4–7.4	6.9	0.3	6.4–7.4
Girls	61 (44.9%)			95 (52.2%)		
Height (cm)	120.0	4.7	113–128	119.2	5.4	111–128
Body weight (kg)	24.3	3.9	19.3–32.8	23.1	3.9	18.2–31.2
Body mass index (kg/m^2)	16.8	2.0	14.2–21.6	16.2	2.0	13.7–20.2
Child's hair mercury (µg/g)	1.90	1.00	0.77–4.06	1.88	1.06	0.68–3.98

There were no significant differences in all items except for body weight and body mass index between the two children groups ($P > 0.05$, Student t test or χ^2 test with Yates correction)

Table 2 Cardiac autonomic indicators and reaction time in Japanese boys and girls at age 7 years (see the Materials and methods)

|	Geometric mean (5 and 95 percentiles)		P value[a]
	75 boys	61 girls	
ECG RR interval variability			
CCV$_{LF}$ (%)	3.61 (1.65–7.02)	4.14 (2.53–7.40)	0.080
CCV$_{HF}$ (%)	3.35 (1.05–8.18)	3.74 (2.01–7.49)	0.259
PSD$_{LF}$ (ms^2)	673 (138–2670)	842 (262–2935)	0.172
PSD$_{HF}$ (ms^2)	579 (41.8–4384)	686 (155–3312)	0.439
%LF	50.0 (30.6–71.7)	51.2 (39.4–71.6)	0.746
LF/HF ratio	1.16 (0.19–6.42)	1.23 (0.42–6.34)	0.760
ECG parameters			
RR interval (ms)	718 (609–854)	700 (606–835)	0.160
QTc interval (ms)	393 (375–419)	394 (375–419)	0.714
Reaction time			
Right hand (ms)	344 (278–477)	346 (281–458)	0.919
Left hand (ms)	365 (292–503)	370 (297–475)	0.740

[a]Student t test

Discussion

In this study, the feasibility to collect umbilical cord samples depended on the original tradition of keeping a piece of the umbilical cord in a paulownia case. As the result, we were able to analyze the effects of prenatal methylmercury exposures among the 136 children. There were no differences in all items, except body weight and BMI, between the children with and without umbilical cord as shown in Table 1. The methylmercury in the cord tissue was considerably lower in 136 children of this study than in patients with Minamata disease, whose levels were between 0.15 and 4.7 µg/g (median 1.63) (Akagi et al. 1998). The hair mercury equivalent concentration can be calculated from the equation of Akagi et al. (1998): maternal hair mercury at parturition (in microgram per gram) = 25.24× Cord-tissue concentration (in µg/g dry weight). Accordingly, the estimated mercury in maternal hair ranges from 0.43 to 9.26 µg/g

(median 2.24) for our subjects, and it was lower than those in the Faroe Islands (0.2–39.1, median 4.5 µg/g) and Seychelles (0.9–25.8, mean 6.5 µg/g) (Grandjean et al. 1992; Myers et al. 2003).

The principal finding of this retrospective study is that methylmercury exposure at parturition was associated with decreased vagal modulation (i.e., low CCV$_{HF}$ and PSD$_{HF}$) of cardiac autonomic function in Japanese children aged 7 years. This agrees with the outcomes of the Faroes cohort at age 14 years (Grandjean et al. 2004), which included the depressed PSD$_{LF}$. The PSD$_{HF}$ was significantly lower in 9 patients with fetal Minamata disease than in 13 control subjects (Oka et al. 2002). In workers, vagal hypoactivity appears to result from occupational exposures to lead, solvents, and vibration (Murata and Araki 1996), and shift work (Ishii et al. 2005), but it is unlikely that the Japanese children have been exposed to such hazardous factors in the environment. However, postnatal mercury exposure in this study was not significantly related to the parasympa-
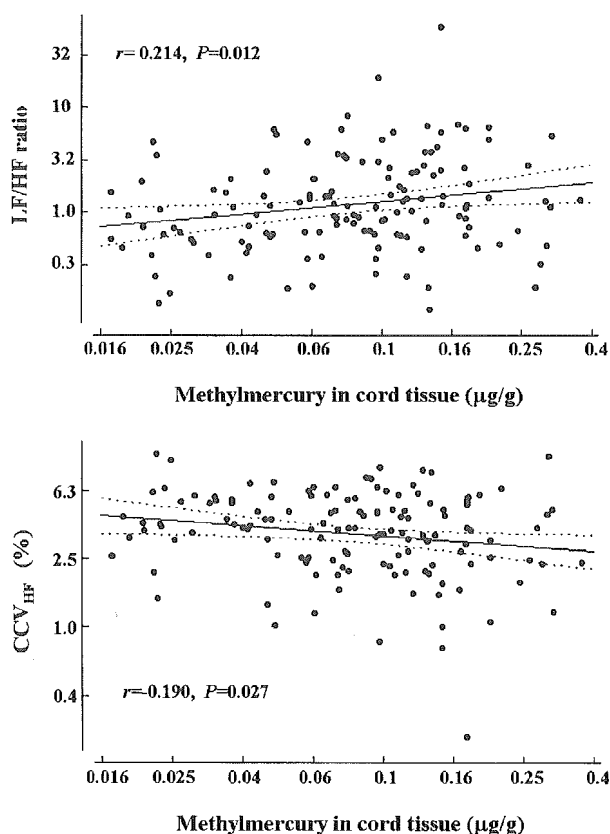
Fig. 1 Pearson product–moment correlations between methylmercury concentrations in cord tissue and cardiac autonomic indicators (CCV_{HF} and LF/HF ratio) in 136 Japanese children. *Dotted lines* represent a 95% confidence limit of linear regression

thetic tone. Though the statistical significance may be reduced by multiple significance test, it is suggested that increased methylmercury exposures may induce depressed parasympathetic activity in the fetus.

The cardiac autonomic function in children of the Faroes study, together with our study, was assessed by one researcher in the same manner (Grandjean et al. 2004), but the two outcomes did not agree in regard to postnatal mercury exposures. In our study, only one standardized regression coefficient between the CCV_{LF} and child's hair mercury was positively significant ($P = 0.041$). By contrast, the CCV_{LF} and PSD_{LF} at age 7 years in the Faroes study were negatively associated with mercury levels in the child's hair (Grandjean et al. 2004); while, any cardiac autonomic endpoints at age 14 years were not affected by postnatal mercury exposures. One possible explanation for this discordance is as follows: prenatal mercury exposures were considerably higher in the Faroes study than in our study, but postnatal mercury exposures were conversely low in the former study (i.e., geometric mean of hair mercury, 0.60 μg/g for 7-year-old children and 0.96 μg/g for 14-year-old children). Also, a significant correlation between the cord blood mercury and 7-year-old child's hair mercury was observed in the Faroes study. For this reason, Grandjean et al. (2004) described that the Faroes study might not allow a clear separation of the effect of exposures at different developmental stage. Apart from autonomic nervous effects of methylmercury, the CCV_{LF} and PSD_{LF} seem to be reduced by chronic high-level exposures to lead and alcohol, but these indicators tend to be unaffected by low-level exposures (Murata and Araki 1991, 1996; Murata et al. 1995). Thus, prenatal methylmercury exposure levels in the Faroes cohort are speculated to have been high enough to affect the sympathetic activity. Taken together, these findings suggest that postnatal exposures to methylmercury at levels of less than 4 μg/g in hair may not influence cardiac autonomic function potently, and such an effect may be reversible, if it exists.

In our research, the LF/HF ratio and %LF, representing a sympathovagal balance (Pagani et al. 1986;

Table 3 Relations of methylmercury in cord tissue (MHg), total mercury in child's hair (HHg) and possible confounders to cardiac autonomic indicators and reaction time in 136 Japanese children at age 7 years: results of multiple regression analysis (see the Materials and methods)

Endpoints	Standardized regression coefficient							R^2
	Log(MHg)	Log(HHg)	Age	Sex	Gestation period	Smoking	Drinking	
ECG RR interval variability								
Log(CCV_{LF})	−0.018	0.179*	−0.157	0.160	−0.048	−0.027	0.036	0.077
Log(CCV_{HF})	−0.196*	0.060	−0.086	0.117	−0.157	−0.014	0.149	0.097
Log(PSD_{LF})	−0.036	0.154	−0.146	0.129	−0.071	−0.031	0.077	0.064
Log(PSD_{HF})	−0.195*	0.041	−0.076	0.089	−0.164	−0.017	0.169*	0.099
%LF	0.205*	0.081	−0.044	0.011	0.138	−0.008	−0.137	0.098
Log(LF/HF ratio)	0.204*	0.091	−0.042	0.011	0.134	−0.008	−0.135	0.098
ECG parameters								
RR interval	−0.078	−0.087	0.029	−0.113	−0.113	−0.025	0.184*	0.081
QTc interval	−0.0002	0.071	−0.012	0.051	0.025	0.120	−0.023	0.020
Reaction time								
Right hand	−0.080	0.060	−0.150	0.004	−0.026	−0.078	0.072	0.041
Left Hand	0.040	−0.017	−0.223*	0.000	−0.015	−0.148	0.038	0.074

*$P < 0.05$

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996), were positively connected with prenatal methylmercury exposure. This finding suggests that a sympathodominant state may have been induced by vagal hypoactivity due to increased mercury exposures, which were within low levels compared to those in the Faroes study (Grandjean et al. 2004). By the way, an increased mortality from ischemic heart disease was found in male Slovenian miners (standardized mortality ratio, 1.66), who had been exposed to inorganic mercury (Boffetta et al. 2001). Also, a high intake of mercury from freshwater fish was involved in an excess risk of myocardial infarction, as well as death from CHD, in eastern Finnish men with hair mercury levels of less than 15.7 μg/g (Salonen et al. 1995) and the risk factor-adjusted odds ratio for myocardial infarction in the highest as compared with the lowest quintile of toenail mercury was 2.16 (95% confidence interval, 1.09–4.29) in an European study (Guallar et al. 2002). On the contrary, American data did not support an association between total mercury exposure and the risk of CHD (Yoshizawa et al. 2002) and the mortality from heart diseases was not increased among 1,483 patients with Minamata disease (Tamashiro et al. 1985). Rather, fish consumption appears to be inversely associated with fatal CHD (He et al. 2004). Apart from mercury exposures, a sympathodominant state and depressed vagal tone have been reported to be associated with the risk for developing CHD (Liao et al. 1997; Lombardi et al. 1987). In spite of several problems related to the type, route, level, and phase of exposures, therefore, an assumption that the increased risk for CHD in adults exposed to mercury may originate from sympathovagal shift of the HRV remains pending. A prospective study of adults with data of HRV parameters, as well as the mercury level, will be necessary to examine this.

Concerning the ECG parameters, cardiac abnormalities such as irregular pulse, ventricular ectopic beats and prolongation of the QT interval, following ethylmercury exposure have been reported in patients who were hospitalized during the Iraqi poisoning epidemic in 1956 (Jalili and Abbasi 1961). In our study, however, no significant link was observed between mercury exposure biomarkers and either the ECG RR interval or QTc. Since the former was not subclinical but clinical cases, this inconsistency would have been attributable to the difference of exposure levels.

Reaction time in the children of our study did not have a close relation to the cord tissue methylmercury or current hair mercury. Likewise, the Seychelles study, with 9-year-old children, failed to find any significant relationship between the mercury in maternal hair at birth and continuous performance task including hit reaction time, attentiveness, and risk-taking (Myers et al. 2003). In contrast, the Faroes study, using a continuous performance test consisting of total number of missed responses and the average reaction time, observed a significant association with the mercury in cord blood in 7- and 14-year-old children (Grandjean et al. 1997, 2002); also, this association was statistically significant considering the effect of PCB (Grandjean et al. 2001). For this reason, we could not derive a conclusion from the available facts with regard to the neuromotor effect of methylmercury. In fact, the type of stimulus presented in the reaction time test differed among three studies.

One limitation of this retrospective study is that co-exposure levels of common contaminants (PCB, chlorinated pesticides, lead, cadmium, etc.), selenium, and omega-3 fatty acids (Grandjean et al. 1992, 2001; Guallar et al. 2002; National Research Council 2000; Sakamoto et al. 2004; Yoshizawa et al. 2002), other than methylmercury could not be determined. The major studies addressing the risk assessment of methylmercury (Davidson et al. 1998; Grandjean et al. 1997, 2004; Kjellström et al. 1989; Myers et al. 2003), except for one Faroes study (Grandjean et al. 2001) did not evaluate the interactions of these coexposures. Such substances in Japan will be assessed in the Tohoku Study of Child Development that is now ongoing (Nakai et al. 2004). Recall bias also could not be avoided more or less, although we confirmed medical records by reference to the maternal and child health booklet. Regarding a possibility that children whose mothers ate too much fish during pregnancy were not included in this study, the children residing in both nonfishing areas and fishing areas in Japan were enrolled as the study population (Murata et al. 2004). Two percent of Japanese reproductive aged females have been reported to exceed the hair mercury level of 5 μg/g (Yasutake et al. 2003), and the estimated hair mercury levels at parturition in our study seem to have shown a similar distribution. In addition, possible confounders such as age, sex, and gestation period were considered in the data analysis.

Another possible limitation is about the HRV. The Task Force on HRV Standards points out that spectral analysis of 24-h recordings may not provide detailed information about autonomic modulation of the RR interval due to the lack of stationarity, and recommends that controlled 5-min recordings processed in the frequency domain be used to study pathophysiologic mechanisms (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). We measured 300 RR intervals in the supine position (i.e., approximately for 4 min in children or for 5 min in adults), and HRV parameters were calculated from consecutive RR intervals with minimal deviation; i.e., the process of RR-interval data extraction for spectral analysis does not depend on examiners. Liao et al. (1997) computed LF- and HF-PSDs of HRV from 2-min beat-to-beat heart rate data in the atherosclerosis risk in communities (ARIC) study. On the other hand, it is also indicated that the power of heart-rate modulation in the frequency range considered to reflect sympathetic activity (PSD_{LF}) tends to be lower than that of the actual activity and may be misinterpreted as a deficiency of sympathetic

outflow (Hilz and Dütsch 2005). In applying spectral analysis with fast Fourier transformation, the ARIC study used records of beat-to-beat data of 2.5 min to capture a few cycles of low-frequency heart rate fluctuation (Liao et al. 1997). We have employed the autoregressive spectral model, and the model does not seem to have caused such problems (Grandjean et al. 2004; Ishii et al. 2005; Murata et al. 1992, 1995, 2005)). Thus, it is suggested that our findings were not heavily influenced by measurement error. In any case, it may make no sense to compare the value of PSD_{LF} or CCV_{LF} between two studies conducted by different researchers.

In conclusion, our data suggest that prenatal methylmercury exposure may be associated with decreased vagal tone or sympathovagal shift in Japanese children, despite the potential limitations involved in the retrospective study. This evidence possesses two significances. The data were provided from another area other than the Faroe Islands and Seychelles. Another is that the usefulness of HRV for subclinical risk assessment on child neurodevelopment was stressed.

References

Akagi H, Grandjean P, Takizawa Y, Weihe P (1998) Methylmercury dose estimation from umbilical cord concentrations in patients with Minamata disease. Environ Res 77:98–103

Akagi H, Castillo ES, Cortes-Maramba N, Francisco-Rivera AT, Timbang TD (2000) Health assessment for mercury exposure among schoolchildren residing near a gold processing and refining plant in Apokon, Tagum, Davao del norte, Philippines. Sci Total Environ 259:31–43

Batten LA, Urbina EM, Berenson GS (2000) Interobserver reproducibility of heart rate variability in children (the Bogalusa heart study). Am J Cardiol 86:1264–1266

Boffetta P, Sällsten G, Garcia-Gómez M, Pompe-Kirn V, Zaridze D, Bulbulyan M, Caballero JD, Ceccarelli F, Kobal AB, Merler E (2001) Mortality from cardiovascular diseases and exposure to inorganic mercury. Occup Environ Med 58:461–466

Clarkson TW (2002) The three modern faces of mercury. Environ Health Perspect 110(Suppl 1):11–23

Dakeishi M, Nakai K, Sakamoto M, Iwata T, Suzuki K, Liu X-J, Ohno T, Kurosawa T, Satoh H, Murata K (2005) Effects of hair treatment on hair mercury—the best biomarker of methylmercury exposure. Environ Health Prev Med 10:208–212

Dalgård C, Grandjean P, Jørgensen PJ, Weihe P (1994) Mercury in the umbilical cord: implication for risk assessment for Minamata disease. Environ Health Perspect 102:548–550

Davidson PW, Myers GJ, Cox C, Axtell C, Shamlaye C, Sloane-Reeves J, Cernichiari E, Needham L, Choi A, Wang Y, Berlin M, Clarkson TW (1998) Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment: outcomes at 66 months of age in the Seychelles child development study. JAMA 280:701–707

Dekker JM, Crow RS, Folsom AR, Hannan PJ, Liao D, Swenne CA, Schouten EG (2000) Low heart rate variability in a 2-minute rhythm strip predicts risk of coronary heart disease and mortality from several causes: the ARIC Study. Circulation 102:1239–1244

Després C, Lamoureux D, Beuter A (2000) Standardization of a neuromotor test battery: the CATSYS system. Neurotoxicology 21:725–735

Finley JP, Nugen ST (1995) Heart rate variability in infants, children and young adults. J Auton Nerv Syst 51:103–108

Grandjean P, Weihe P, Jørgensen PJ, Clarkson T, Cernichiari E, Viderø T (1992) Impact of maternal seafood diet on fetal exposure to mercury, selenium, and lead. Arch Environ Health 47:185–195

Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, Murata K, Sørensen N, Dahl R, Jørgensen PJ (1997) Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol Teratol 19:417–428

Grandjean P, Weihe P, Burse VW, Needham LL, Storr-Hansen E, Heinzow B, Debes F, Murata K, Simonsen H, Ellefsen P, Budtz-Jørgensen E, Keiding N, White RF (2001) Neurobehavioral deficits associated with PCB in 7-year-old children prenatally exposed to seafood neurotoxicants. Neurotoxicol Teratol 23:305–317

Grandjean P, White RF, Debes F, Weihe P, Letz R (2002) NES2 continuous performance test results obtained by methylmercury-exposed children at age 7 and 14 years. In: Abstract book of 8th international symposium: neurobehavioral methods and effects in occupational and environmental health. Institute of Occupational Health and Industrial Hygiene, University of Brescia, Brescia, p 136

Grandjean P, Murata K, Budtz-Jørgensen E, Weihe P (2004) Cardiac autonomic activity in methylmercury neurotoxicity: 14-year follow-up of a Faroese birth cohort. J Pediatr 144:169–176

Guallar E, Sanz-Gallardo I, van't Veer P, Bode P, Aro A, Gomez-Aracena J, Kark JD, Riemersma RA, Martin-Moreno JM, Kok FJ, Heavy Metal, Myocardial Infarction Study Group (2002) Mercury, fish oils, and the risk of myocardial infarction. N Engl J Med 347:1747–1754

Harada M (1995) Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. Crit Rev Toxicol 25:1–24

He K, Song Y, Daviglus ML, Liu K, Van Horn L, Dyer AR, Greenland P (2004) Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies. Circulation 109:2705–2711

Hilz MJ, Dütsch M (2005) Quantitative studies of autonomic function. Muscle Nerve (in press, available online 17 June 2005)

Igata A (1993) Epidemiological and clinical features of Minamata disease. Environ Res 63:157–169

Ishii N, Dakeishi M, Iwata T, Sasaki M, Murata K (2005) Cardiac autonomic imbalance in female nurses with shift work. Auton Neurosci 122:94–99

Iwasaki Y, Sakamoto M, Nakai K, Oka T, Dakeishi M, Iwata T, Satoh H, Murata K (2003) Estimation of daily mercury intake from seafood in Japanese women: akita cross-sectional study. Tohoku J Exp Med 200:67–73

Jalili MA, Abbasi AH (1961) Poisoning by ethyl mercury toluene sulphonanilide. Br J Ind Med 18:303–308

Kjellström T, Kennedy P, Wallis S (1989) Physical and mental development of children with prenatal exposure to mercury from fish. Stage 2, interviews and psychological tests at age 6 (report 3642). National Swedish Environmental Protection Board, Stockholm

Liao D, Cai J, Rosamond WD, Barnes RW, Hutchinson RG, Whitsel EA, Rautaharju P, Heiss G (1997) Cardiac autonomic function and incident coronary heart disease: a population-based case-cohort study—the ARIC study. Am J Epidemiol 145:696–706

Lombardi F, Sandrone G, Pernpruner S, Sala R, Garimoldi M, Cerutti S, Baselli G, Pagani M, Malliani A (1987) Heart rate variability as an index of sympathovagal interaction after acute myocardial infarction. Am J Cardiol 60:1239–1245

Massin M, von Bernuth G (1997) Normal ranges of heart rate variability during infancy and childhood. Pediatr Cardiol 18:297–302

Murata K, Araki S (1991) 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 20:663–671

Murata K, Araki S (1996) Assessment of autonomic neurotoxicity in occupational and environmental health as determined by ECG R–R interval variability: a review. Am J Ind Med 30:155–163

Murata K, Dakeishi M (2005) Neurodevelopmental effects from prenatal exposure to methylmercury in the Seychellois and Faroes cohorts and the critical concentration: a review. Jpn J Hyg 60:4–14

Murata K, Yano E (2002) Medical statistics for evidence-based medicine with SPBS user's guide. Nankodo Publisher, Tokyo

Murata K, Landrigan PJ, Araki S (1992) Effects of age, heart rate, gender, tobacco and alcohol ingestion on R–R interval variability in human ECG. J Auton Nerv Syst 37:199–206

Murata K, Araki S, Yokoyama K, Nomiyama K, Nomiyama H, Tao YX, Liu SJ (1995) Autonomic and central nervous system effects of lead in female glass workers in China. Am J Ind Med 28:233–244

Murata K, Weihe P, Renzoni A, Debes F, Vasconcelos R, Zino F, Araki S, Jørgensen PJ, White RF, Grandjean P (1999a) Delayed evoked potentials in children exposed to methylmercury from seafood. Neurotoxicol Teratol 21:343–348

Murata K, Yano E, Shinozaki T (1999b) Impact of shift work on cardiovascular functions in a 10-year follow-up study. Scand J Work Environ Health 25:272–277

Murata K, Sakamoto M, Nakai K, Weihe P, Dakeishi M, Iwata T, Liu XJ, Ohno T, Kurosawa T, Kamiya K, Satoh H (2004) Effects of methylmercury on neurodevelopment in Japanese children in relation to the Madeiran study. Int Arch Occup Environ Health 77:571–579

Murata K, Yano E, Hashimoto H, Karita K, Dakeishi M (2005) Effects of shift work on QTc interval and blood pressure in relation to heart rate variability. Int Arch Occup Environ Health 78:287–292

Myers GJ, Davidson PW, Cox C, Shamlaye CF, Palumbo D, Cernichiari E, Sloane-Reeves J, Wilding GE, Kost J, Huang LS, Clarkson TW (2003) Prenatal methylmercury exposure from ocean fish consumption in the Seychelles child development study. Lancet 361:1686–1692

Nakai K, Suzuki K, Oka T, Murata K, Sakamoto M, Okamura K, Hosokawa T, Sakai T, Nakamura T, Saito Y, Kurokawa N, Kameo S, Satoh H (2004) The Tohoku study of child development: a cohort study of effects of perinatal exposures to methylmercury and environmentally persistent organic pollutants on neurobehavioral development in Japanese children. Tohoku J Exp Med 202:227–237

National Research Council (2000) Toxicological effects of methylmercury. National Academy Press, Washington

Oka T, Matsukura M, Okamoto M, Harada N, Kitano T, Miike T, Futatsuka M (2002) Autonomic nervous functions in fetal type Minamata disease patients: assessment of heart rate variability. Tohoku J Exp Med 198:215–221

Pagani M, Lombardi F, Guzzetti S, Rimoldi O, Furlan R, Pizzinelli P, Sandrone G, Malfatto G, Dell'Orto S, Piccaluga E, Turiel M, Baselli G, Cerutti S, Malliani A (1986) Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympathovagal interaction in man and conscious dog. Circ Res 59:178–193

Sakamoto M, Kubota M, Liu XJ, Murata K, Nakai K, Satoh H (2004) Maternal and fetal mercury and n-3 polyunsaturated fatty acids as a risk and benefit of fish consumption to fetus. Environ Sci Technol 38:3860–3863

Salonen JT, Seppänen K, Nyyssönen K, Korpela H, Kauhanen J, Kantola M, Tuomilehto J, Esterbauer H, Tatzber F, Salonen R (1995) Intake of mercury from fish, lipid peroxidation, and the risk of myocardial infarction and coronary, cardiovascular, and any death in Eastern Finnish men. Circulation 91:645–655

Silvetti MS, Drago F, Ragonese P (2001) Heart rate variability in healthy children and adolescents is partially related to age and gender. Int J Cardiol 81:169–174

Sørensen N, Murata K, Budtz-Jørgensen E, Weihe P, Grandjean P (1999) Prenatal methylmercury exposure as a cardiovascular risk factor at seven years of age. Epidemiology 10:370–375

Tamashiro H, Arakaki M, Akagi H, Futatsuka M, Roht LH (1985) Mortality and survival for Minamata disease. Int J Epidemiol 14:582–588

Task Force of the European Society of Cardiology, the North American Society of Pacing and Electrophysiology (1996) Heart rate variability: standards of measurement, physiological interpretation, and clinical use. Circulation 93:1043–1065

Yasutake A, Matsumoto M, Yamaguchi M, Hachiya N (2003) Current hair mercury levels in Japanese: survey in five districts. Tohoku J Exp Med 199:161–169

Yoshizawa K, Rimm EB, Morris JS, Spate VL, Hsieh CC, Spiegelman D, Stampfer MJ, Willett WC (2002) Mercury and the risk of coronary heart disease in men. N Engl J Med 347:1755–1760