Discussion

Several early studies demonstrated the adverse effects of maternal smoking during pregnancy on motor function in the neonatal period. Law et al. (2003) examined the association of maternal smoking with infant neurobehavioral status 48 hours after birth. They found that infants of the smoking group showed more signs of stress and were hypertonic and excitable. Fried et al. (1987) [13] examined the association of infant neurological status with maternal cigarette smoking, marijuana use, and alcohol use. They assessed the neurological status using the Prechtl Neurological Examination at 9 and 30 days after birth, and found that maternal smoking during pregnancy was associated with hypertonicity and increased nervous system excitation, particularly at 30 days. Dempsey et al. (2000) [14] assessed neonatal neurobehavioral status with neurological examination, and found an association of neonatal hypertonicity with maternal smoking. In our previous report we found an association between maternal smoking during pregnancy and a lower score for muscle tone [23]. In the present study, we found significant associations between maternal smoking during pregnancy and lower scores for 'pull to sit' and 'defensive movements'. 'Pull to sit' evaluates muscle tone when the infant is pulled to sit, and 'defensive movements' evaluates the coordination of movement when a cloth is put on the infant's face. These 2 behavioral items, as well as muscle tone, are included in the motor cluster, and indicate muscle tone and motor coordination. Our findings appear to be in line with previous studies, and suggest that maternal smoking during pregnancy adversely affects neurobehavioral status, especially motor function, during the neonatal period.

However, there were no significant associations of maternal smoking during pregnancy with the results of the FTII and the BSID-II in children 7 and 18 months of age. Two possible explanations for this result should be considered. First, because of the interaction of postnatal environmental factors, the effects seen in the neonatal period become more difficult to observe. Indeed, several studies reported that the effects of maternal smoking during pregnancy virtually disappeared even after controlling for the quality of the home environment and the SES. Olds et al. (1994a) [34] examined the associations between maternal smoking during pregnancy and offspring neurobehavioral development during the first 4 years. After adjusting for covariates such as the quality of the home environment, the SES, and mother's IQ, maternal smoking was not associated with the BSID at 1 year of age or the Cattel scale at 2 years of age. Olds et al. (1994b) [35] pointed out the possibility that adequate quality of the home environment can offset the impairment in cognitive function associated with maternal smoking during pregnancy. Fried et al. (1988) [17] employed the BSID at 12 and 24 months of age, and examined the associations between maternal cigarette smoking, marijuana use and alcohol use. They found significant associations between maternal smoking and a lower MDI at 12 months of age and altered responses to auditory items at 12 and 24 months. However, at 24 months, the strong relationship of postnatal environmental factors with cognitive outcomes resulted in loss of the statistical significance of data. Trasti et al. (1999) [21] examined the association of maternal smoking in pregnancy with mental and motor development at age 1 and 5 years, respectively. No significant associations were found between maternal smoking and the BSID-II at 1 year of age. Children of smokers showed lower Whecsler Preschool and Primary Scale of Intelligence

Revised (WPPSI-R) scores at 5 years of age than children of nonsmokers. However, after controlling for maternal educational status, this difference was not statistically significant. Our findings may be in line with these studies. Furthermore, in the present study, positive associations were found between the EES and the BSID-II. The EES evaluates the quality of the home environment regarding child rearing. Although social class and economic status tend to have positive correlations to the home environment generally, no significant association was found between household income and the FTII and the BSID-II in the present study. Moreover, there was no statistically significant correlation between household income and the EES (data not shown). These findings suggest that the quality of the child-rearing environment has a great influence on child development, independent of the socioeconomic status. Additionally, the plausible adverse effects due to maternal smoking during pregnancy can be reduced by favorable environmental conditions such as compensatory postnatal caregiving. Another explanation for this result is that the effects of maternal smoking during pregnancy on development were unrelated to the domains of development measured by the FTII and the BSID-II. There is a possibility that the effects of maternal smoking could be detected in the NBAS, but not in the FTII and the BSID-II. To examine this possibility, correlations among the 3 developmental measurements were examined (Table 5). The 2 behavioral items of the NBAS, which were found to be significant in the analysis of maternal smoking, were not correlated with any of the indices in the FTII and the BSID-II. These results suggest that maternal smoking adversely affects neonatal neurobehavioral status; however, these effects could not be detected with the assessment tools we used in infancy.

Many studies have linked reductions of children's behavioral functions to maternal smoking during pregnancy [36][37][38][39]. Some of these studies indicated that the connection between maternal smoking and children's maladaptive behaviors was more consistent than the data about cognitive functions. One of the studies of the behavioral effects of maternal smoking during pregnancy assessed 1,377 pairs of twins 2 to 3 years of age using the Child Behavior Checklist (CBCL). Maternal smoking was associated with a significant increase in externalizing (e.g., oppositional, aggressive, overactive) but not internalizing (e.g., withdrawn, depressed, anxious) behavioral problems in both first and second born twins [37]. Wasserman et al. (1999) [38] replicated this finding in 191 children 4 to 5 years old, showing an association between maternal smoking and higher scores on all CBCL subscales except anxious/depressed and somatic complaints. These findings suggest that the effects of maternal smoking during pregnancy might appear in a child's behavioral domain with regard to attention, hyperactivity and maladaptive behavior.

Table 5. Correlations and partial correlations among the NBAS, FTII, and BSID-II

	7 months						18 months			
	% of time looking at a novel target		MDI		PDI		MDI		PDI	
	r	Partial r	r	Partial r	r	Partial r	r	Partial r	r	Partial r
Motor cluster	-0.02	-0.02	-0.04	-0.01	0.02	0.01	0.02	0.02	0.02	0.03
Pull to sit	-0.05	-0.08	-0.08	-0.06	-0.03	-0.01	-0.07	-0.03	-0.06	-0.06
Defensive movement	0.02	0.01	0.00	0.00	0.05	-0.00	0.07	-0.08	0.10	0.07

n.s

% of time looking at a novel target, the score of the Fagan Test of Infant Intelligence (FTII); MDI, BSID-II Mental Developmental Index; PDI, BSID-II Psychomotor Developmental Index; EES, the Evaluation of Environmental Stimulation; the Raven score, the raw score of the Raven Standard Matrices.

Spearman's r.

For calculating partial correlation, the Raven score, the EES score, and household income were used as covariates.

There are some limitations in this study. First, the data on maternal smoking habits were collected using a self-administered questionnaire. Objective measurements of salivary, serum, and urine cotinine levels were not perfomed. Although several studies found moderate correlations between self-reported smoking habits and biomarkers of cigarette exposure [40][41][42], misclassification of the maternal cigarette use cannot be ruled out. Second, compared with reports from the Japan Ministry of Health, Labour, and Welfare, in which the percentage of women who smoked during pregnancy was estimated to be 10.0% in 2000 [1], the percentage of smokers in the present study was low (3.4%). The small number of smokers may be a limitation of this study. As such, interpretation of the findings in the present study may require further consideration.

Finally, the present study reveals the adverse effects of maternal smoking during pregnancy on neurobehavioral status of offspring in their neonatal period. However, these effects were not observed on cognitive functions of offspring in their infancy. A significant association of the postnatal child rearing environment with child development was suggested. Several studies have indicated poorer behavioral function of offspring in their childhood due to maternal smoking during pregnancy. The present study is a prospective cohort study; evaluations of the long-term effects of maternal smoking during pregnancy on development of offspring from diverse viewpoints will be continued. The clinical significance of the negative change in motor function in the neonatal period is less well understood; it may have possible links to behavioral function in childhood. In the future we will readdress this health issue considering the interaction of the postnatal environmental factors with child development.

Conclusion

The present study examined the associations between maternal smoking during pregnancy and offspring development, considering the interaction of postnatal environmental factors. The results indicated that adverse effects of maternal smoking during pregnancy on neurobehavioral development of offspring could be detected in the neonatal period; however, these effects could not be found in their later infancy. The quality of the postnatal child-rearing environment was positively associated with the BSID-II, suggesting that the postnatal environment has a great influence on child development. Further studies examining the effects of maternal smoking on offspring development should take into consideration the roles of postnatal environmental factors in the interactions that determine child development.

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ASSOCIATION OF MATERNAL SMOKING DURING PREGNANCY AND INFANT NEUROBEHAVIORAL STATUS[1,2]

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Summary.—The association of maternal smoking during pregnancy with neurobehavioral status was examined in 344 Japanese infants. Based on a questionnaire, their mothers were classified into three groups, Nonsmokers, Exsmokers, and Smokers. The Neonatal Behavioral Assessment Scale was administered three days after birth. Among the three groups, on the seven clusters and their 28 behavioral subscales there were no significant differences. The infants of Smokers had lower scores than those of Exsmokers and Nonsmokers on two behavioral items, general tone and peak of excitement. General tone remained significant after adjustment for covariates.

Although the number of cigarette smokers in Japan declined during the 1990s, the numbers of women who smoked increased. The percentage of women who smoked during pregnancy was 5.6% in 1990 and increased to 10.0% in 2000 (Maternal and Child Health Division, Equal Employment,

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Children and Families Bureau, Ministry of Health, Labour and Welfare, 2000). Cigarette smoking of women, especially smoking during pregnancy, continues to be a significant public health concern in Japan.

Maternal smoking during pregnancy is well known to have various effects on the fetus and infant. Children of smoking mothers are often born with lower birth weight or lower gestational age (Matsubara, Kida, Tamakoshi, Wakai, Kawamura, & Ohno, 2000; Weitzman, Byrd, Aligne, & Moss, 2002). For instance, Weitzman, *et al.* (2002) reported that the infants born to smoking mothers weigh an average of 150 to 300 gm less than infants born to nonsmoking mothers. In addition, there is an increase in the risk of premature labour, intrauterine growth retardation, and perinatal mortality as the result of cigarette use in the prenatal period (Mattson, Calarco, Chambers, & Jones, 2002).

Although there are many reports about maternal smoking and infant neurobehavioral status, the effects of maternal smoking on infant neurobehavioral status are poorly understood. Several studies indicated maternal smoking adversely affected infant muscle tone (Fried, Watkinson, Dillon, & Dulberg, 1987; Dempsey, Hajnal, Partridge, Jacobson, Good, Jones, & Ferriero, 2000; Law, Stroud, LaGasse, Niaura, Liu, & Lester, 2003). In a study using the NICU Network Neurobehavioral Scale (NNNS), infants of the smoking group showed more signs of stress or abstinence and were hypertonic and excitable (Law, *et al.*, 2003). A study in which the Prechtl Neurological Examination was used yielded an association between maternal smoking during pregnancy and infant hypertonicity and increased nervous system excitation (Fried, Watkinson, & Gray, 1998). In a study using neurological examination, maternal smoking was significantly associated with infants' muscle-tone abnormalities (Dempsey, *et al.*, 2000). On the other hand, studies in which the Neonatal Behavioral Assessment Scale was used yielded an association of a lower orientation cluster but not muscle tone with maternal smoking (Saxton, 1978; Oyamade, Cole, Johnson, Knight, Westney, Laryea, Hill, Cannon, Fomufod, Westney, Jones, & Edwards, 1994). In other studies using the Neonatal Behavioral Assessment Scale, smoking was not associated with neonatal neurobehavioral status after adjustment for covariates (Richardson, Day, & Taylor, 1989), although effects of alcohol and caffeine intake during pregnancy were found (Jacobson, Fein, Jacobson, Schwartz, & Dowler, 1984). Espy, Riese, and Francis (1997) found associations of negative changes in motor development and vigor cluster with maternal cocaine exposure and alcohol drinking during pregnancy but not an association of neonatal neurobehavioral status with maternal smoking. Although several studies yielded effects of maternal smoking on infants' muscle tone, the results obtained so far are controversial. There have been no reports on the association of infant neurobehavioral status with maternal smoking in Japan.

In an ongoing longitudinal prospective cohort study, the Tohoku Study of Child Development, the effects of perinatal exposure to environmentally persistent organic pollutants or heavy metals on child development are being examined (Nakai, Suzuki, Oka, Murata, Sakamoto, Okamura, Hosokawa, Sakai, Nakamura, Saito, Kurokawa, Kameo, & Satoh, 2004). Since maternal smoking is thought to be an important confounding factor in children's neurobehavioral development, the effects of maternal smoking on child development were independently analyzed. In the present study, the relation of infants' neurobehavioral status with maternal smoking during pregnancy was examined.

Method

Subjects

The subjects were 344 singleton babies and their mothers. The healthy pregnant women were registered between January 2001 and August 2002 at obstetrical wards of two hospitals in Sendai, Japan. They were registered with their informed consent for participation in this study according to guidelines established by the ethical committee of Tohoku University Graduate School of Medicine. Mean maternal age at the time of delivery was 30.6 yr. (SD= 0.8). The infants were 179 boys and 165 girls. They were full-term (36 to 42 weeks of gestation), with birth weight of over 2500 gm and without congenital anomalies or diseases.

Procedure and Measures

Infant neurobehavioral examination.—Infant neurobehavioral status was examined using the Neonatal Behavioral Assessment Scale (Brazelton, 1973). The scale combines neurological items with an extended behavioral repertoire of the infant in an interactional process (Brazelton & Nugent, 1995). The basic score is composed of 28 behavioral items and 18 reflex items. To compare the evaluations of the infants' behavior, the items that interact in similar ways have been classified into seven clusters describing global functions (Lester, Als, & Brazelton, 1982): (1) Habituation which includes items assessing an infant's reactivity to stimulation from a rattle, bell, light, and mild pin prick, followed by response decrement while in a light sleep state; (2) Orientation which includes attention to visual and auditory stimuli during alert states; (3) Motor which measures the quality of muscle tone and movement; (4) Range of state which includes items related to arousal; (5) Regulation of state which reflects the quality of the infant's responses when aroused and ability to control arousal in response to environmental stimulation; (6) Autonomic stability which includes items assessing physiologic responses to stress; and (7) Reflexes which reflects the number of abnormally elicited reflexes.

Neurobehavioral status was assessed with the Neonatal Behavioral Assessment Scale when the infants were three days old. All examinations took place in a quiet room under predetermined lighting and temperature conditions. Examiners were trained and certified to administer the scale at the Training Center for the Neonatal Behavioral Assessment Scale in Nagasaki University School of Medicine in Japan. Reliability confirmation was conducted throughout data collection to maintain 90% agreement for measurements.

Maternal questionnaire and smoking classification.—Mothers were asked about their characteristics, including smoking and alcohol consumption during pregnancy, on the fourth day after delivery. Maternal smoking was divided into four categories; (1) Never smoked, (2) Ceased to smoke in the past, (3) Ceased to smoke on discovery of pregnancy, and (4) Smoked throughout pregnancy. When the statistical analyses were conducted, mothers were classified into three groups by their smoking habits based on the questionnaire; Nonsmokers including those in (1) and (2), Exsmokers who ceased (3), and Smokers who ceased (4).

Statistical Analysis

For three-group comparisons, homogeneity of variance was assessed by the Levene test. Parametric comparisons used analysis of variance. *Post hoc* analysis was performed with Tukey's *HSD* test. When the Levene test indicated significant differences among groups, a nonparametric test, the Kruskal-Wallis test, was performed. The *post hoc* test employed the Mann-Whitney test. Comparisons of categorical variables were performed by Fisher's exact test. On the Neonatal Behavioral Assessment Scale, seven clusters and their 28 behavioral subscales were used for analysis.

Adjusted mean scores on the Neonatal Behavioral Assessment Scale were compared among groups. To adjust for covariates, analysis of covariance was applied, *post hoc* followed by Tukey's *HSD* test. Potential covariates were selected from maternal and infant characteristics (Table 1 below) by four examiners. There were moderate to strong correlations between birth weight and birth head circumference (Pearson $r = .54$, $p < .01$), and birth weight and birth length (Pearson $r = .71$, $p < .01$). In consideration of multicollinearity, birth head circumference and birth length were excluded from the covariates. All analyses were done using the statistical program JMP, Version 5, for Macintosh computers (SAS Institute Japan, Inc.). Significance was set at 5%.

Results

Status of Maternal Smoking

Twelve of 344 (3.4%) mothers smoked throughout pregnancy. Of the 332 mothers who did not smoke, 44 were Exsmokers and 288 were Nonsmokers. Smokers reported that they smoked an average of 13.0 ($SD=6.5$, range 2–20) cigarettes per day during pregnancy.

Maternal and Infant Characteristics

Table 1 shows the maternal and infant characteristics. Mothers of the smoking group were younger than in the other two groups ($F_{2,341}=6.40$, $p<.01$). Infants' characteristics did not differ among the three groups of mothers.

TABLE 1
MATERNAL AND INFANT CHARACTERISTICS

	Maternal Smoking During Pregnancy						F	η^2
	No				Yes			
	Nonsmokers ($n=288$)		Exsmokers ($n=44$)		Smokers ($n=12$)			
	M	*SD*	*M*	*SD*	*M*	*SD*		
Maternal Prenancy								
Age†	32.2	4.3	30.4	6.1	29.1	4.3	6.40	.04
Alcohol use (no/yes)[a]	225/63		30/14		4/8			
Delivery type (spontaneous/Cesarean)[a]	233/55		40/14		8/4			
Parity (first/other)[a]	144/144		40/14		6/6			
Infant								
Sex (M/F)[a]	145/143		28/16		6/6			
Gestational age, wk.	39.4	1.4	39.7	1.3	39.2	0.7	1.34	.008
Birth weight, gm	3018.0	380.0	3108.0	380.0	3184.0	415.4	1.44	.008
Birth length, cm	48.9	2.2	49.3	1.8	49.0	2.3	0.59	.003
Head circumference, cm	33.5	1.4	33.4	1.3	33.9	1.5	0.87	.005
Apgar, 1 min.	8.0	0.9	7.9	0.7	8.3	0.5	1.50	.009

[a]Fisher's exact test. †$p<.01$ by analysis of variance.

Neonatal Behavioral Assessment Scale Performance

Table 2 shows the unadjusted mean Neonatal Behavioral Assessment Scale scores for the three groups. There were no significant differences among groups on the seven clusters. The 29 behavioral items were analyzed next. On the inanimate auditory item, the infants of Exsmokers showed a higher mean score than the Nonsmokers ($F_{2,285}=3.42$, $p<.05$). For the general tone item, significant differences among groups were found ($F_{2,305}=3.14$, $p<.05$). For the peak of excitement, the infants of Smokers had a lower mean score than the other two groups ($F_{2,305}=3.51$, $p<.01$).

Table 3 shows the adjusted mean Neonatal Behavioral Assessment Scale scores of the three groups. There were no significant differences for any of the seven clusters. Among the behavioral items, general tone was still statistically significant with covariates controlled ($F_{2,290}=3.33$, $p<.05$). Peak of excitement, cuddliness, and inanimate auditory orientation were not significant after adjustment for covariates.

TABLE 2

NEONATAL BEHAVIORAL ASSESSMENT SCALE UNADJUSTED MEAN SCORES OF THREE GROUPS

Cluster and Behavioral Item	Nonsmokers (n=288)		Exsmokers (n=44)		Smokers (n=12)		F	η^2
	M	SD	M	SD	M	SD		
Habituation	6.57	1.38	6.65	1.33	6.36	1.29	0.20	.001
Response decrement to light	5.82	1.94	5.77	1.94	5.00	2.19	0.98	.007
Response decrement to rattle	7.04	1.51	7.16	1.25	6.91	1.36	0.57	.005
Response decrement to bell	7.82	2.12	8.09	2.33	8.13	1.97	0.08	.001
Response decrement to foot stimulation	6.25	2.16	6.14	1.96	6.00	1.50	0.11	.001
Orientation	4.57	1.13	4.91	1.18	4.40	1.42	1.94	.01
Animate visual	4.85	1.34	4.62	1.45	4.00	0.93	0.75	.005
Animate visual and auditory	5.05	1.32	5.30	1.28	4.42	1.13	1.56	.01
Inanimate visual	3.90	1.50	4.33	1.73	4.04	1.17	1.66	.02
Inanimate visual and auditory	5.23	1.55	5.58	1.59	4.88	1.36	1.21	.009
Animate auditory	4.33	1.73	4.48	1.88	4.50	2.33	0.16	.001
Inanimate auditory*	4.50	1.33	5.09	1.63	4.44	2.13	3.42	.02
Alertness	4.30	1.28	4.74	1.67	4.33	1.50	1.99	.01
Motor	4.63	0.65	4.59	0.69	4.55	0.89	0.18	.001
General tone*	5.06	0.99	4.87	1.03	4.36	1.12	3.14	.02
Motor maturity	4.16	1.14	4.28	1.06	4.82	1.25	1.95	.01
Pull to sit	4.91	1.21	4.75	1.25	4.64	1.69	0.28	.002
Defensive movements	4.76	1.64	4.70	1.60	4.82	1.99	0.04	.000
Activity	4.29	0.70	4.28	0.77	4.09	0.70	0.42	.003
Range of State	3.88	0.73	3.84	0.77	3.77	0.95	0.13	.001
Peak of excitement*	3.20	1.12	3.32	0.93	2.36	1.03	3.51	.02
Rapidity of build-up	4.14	1.51	4.04	1.74	4.09	1.45	0.07	.000
Irritability	4.34	1.63	4.31	1.89	5.00	1.49	0.92	.006
Lability of state	3.83	0.80	3.79	0.95	3.82	1.33	0.04	.000
Regulation of State	4.23	1.16	4.48	1.10	4.29	1.92	0.91	.006
Cuddliness	4.64	1.33	4.72	1.75	3.64	1.50	2.85	.02
Consolability	4.44	1.51	4.95	1.49	4.13	2.95	2.01	.01
Self-quieting	4.13	2.56	4.36	2.48	4.36	3.35	0.21	.001
Hand to mouth	3.66	2.33	3.91	2.45	4.36	2.87	0.67	.004
Autonomic Stability	5.69	1.00	5.92	1.02	6.09	0.88	0.65	.004
Tremulousness	5.31	1.82	5.66	2.05	6.27	1.67	1.96	.01
Startles	6.91	1.48	7.09	1.49	7.36	1.03	0.72	.01
Lability of skin color	4.86	0.93	5.02	0.85	4.64	0.92	0.24	.002
Reflex	2.65	1.85	2.89	2.13	3.18	2.44	0.65	.004

*$p < .05$ by analysis of variance.

DISCUSSION

Several studies have found negative associations between maternal smoking and neonatal muscle tone. Law, *et al.* (2003) examined the association of maternal smoking during pregnancy with infant neurobehavioral status, using the NICU Network Neurobehavioral Scale at 48 hours after birth. They stated that infants of their smoking group showed more signs of

TABLE 3

Neonatal Behavioral Assessment Scale Adjusted Mean Scores of Three Groups

Cluster and Behavioral Item	Nonsmokers ($n=288$)		Exsmokers ($n=44$)		Smokers ($n=12$)		F	η^2
	M	*SD*	*M*	*SD*	*M*	*SD*		
Habituation	6.41	0.13	6.50	0.22	6.63	0.40	0.55	.004
Response decrement to light	5.53	0.18	5.29	0.31	5.27	0.57	0.23	.002
Response decrement to rattle	7.00	0.21	7.19	0.36	7.51	0.66	0.94	.009
Response decrement to bell	7.75	0.18	7.98	0.29	8.05	0.54	0.71	.005
Response decrement to foot stimulation	6.11	0.22	6.08	0.36	6.00	0.72	0.05	.001
Orientation	4.57	0.11	4.93	0.18	4.49	0.36	2.76	.02
Animate visual	4.40	0.13	4.66	0.21	4.08	0.44	1.33	.01
Animate visual and auditory	4.91	0.13	5.24	0.22	4.57	0.48	2.19	.02
Inanimate visual	3.80	0.19	4.33	0.31	4.04	0.66	1.71	.02
Inanimate visual and auditory	5.35	0.17	5.66	0.27	4.85	0.56	1.64	.01
Animate auditory	4.32	0.17	4.48	0.27	4.78	0.56	0.65	.005
Inanimate auditory	4.62	0.14	5.14	0.23	4.57	0.46	3.08	.02
Alertness	4.34	0.15	4.77	0.23	4.39	0.47	2.53	.02
Motor	4.66	0.06	4.57	0.10	4.58	0.19	0.32	.002
General tone*	5.11	0.10	4.88	0.16	4.45	0.30	3.33	.02
Motor maturity	4.24	0.10	4.33	0.16	4.75	0.31	1.34	.009
Pull to sit	4.79	0.12	4.68	0.20	4.62	0.37	0.21	.001
Defensive movements	4.85	0.16	4.70	0.27	4.96	0.50	0.04	.001
Activity level	4.27	0.07	4.24	0.12	4.06	0.22	0.49	.003
Range of State	3.88	0.07	3.80	0.12	3.91	0.23	0.03	.001
Peak of excitement	3.26	0.11	3.35	0.18	2.58	0.33	2.24	.02
Rapidity of build-up	4.14	0.15	3.84	0.25	4.02	0.48	0.19	.002
Irritability	4.45	0.17	4.28	0.20	5.32	0.54	1.23	.008
Lability of state	3.69	0.09	3.72	0.14	3.86	0.27	0.24	.002
Regulation of State	4.37	0.12	4.61	0.20	4.44	0.37	0.40	.003
Cuddliness	4.73	0.14	4.78	0.23	3.78	0.43	2.85	.02
Consolability	4.68	0.17	5.17	0.29	4.41	0.58	1.65	.01
Self-quieting	4.08	0.26	4.42	0.44	4.31	0.81	0.13	.001
Hand to mouth	3.91	0.24	4.06	0.39	4.58	0.74	0.46	.003
Autonomic Stability	5.72	0.10	5.85	0.16	5.91	0.31	0.33	.002
Tremulousness	5.53	0.17	5.75	0.28	5.75	0.51	0.31	.002
Startles	6.79	0.15	6.77	0.24	7.20	0.46	0.54	.004
Lability of skin color	6.79	0.15	6.77	0.24	7.20	0.46	0.11	.001
Reflex	3.09	0.18	3.40	0.31	3.37	0.57	0.49	.003

*$p<.05$ by analysis of covariance.

stress or abstinence and were hypertonic and excitable. Fried, *et al.* (1987) examined the association of infant neurological status with maternal cigarette smoking, marijuana use, and alcohol use. They assessed the neurological status by Prechtl Neurological Examination at 9 and 30 days after birth and found that prenatal cigarette exposure was associated with hypertonicity and increased nervous system excitation, particularly at 30 days. Dempsey, *et al.*

(2000) assessed neonatal neurobehavioral status with a neurological examination and reported an association of neonatal hypertonicity with maternal smoking. Our observation of the association between maternal smoking during pregnancy and a lower score for general tone may be in line with these studies. Infants' muscle tone is thought to indicate the maturity of the central nervous system (Prechtl, 1977).

Many reports have described the association of maternal smoking with birth weight (Andres & Day, 2000; Matsubara, *et al.*, 2000; Weitzman, *et al.*, 2002). However, no association of maternal smoking and birth weight was noted here. In this cohort study were included only infants who were full-term (36–42 weeks of gestation) and had a birth weight of over 2500 gm. Although selection bias is possible given to exclusion of lower-birth weight infants, there were 22 infants who were excluded due to lower birth weight and shorter gestational age, none of whose mothers had been smoking during pregnancy. Therefore, this type of selection bias would not be expected. Moreover, Law, *et al.* (2003) reported a negative association of maternal smoking with neurobehavioral status, including muscle tone, without finding one with birth weight. In addition, the nicotine in cigarettes is thought to act as a neuroteratogen that interferes with fetal development (Slotkin, 1998). This suggests that maternal smoking adversely affects neurobehavioral development at smoking doses which do not affect growth parameters such as birth weight; however, these results suggested that maternal smoking directly affects infant neurobehavioral status.

Although Japan Ministry of Health, Labour and Welfare reported that the prevalence of women who smoked during pregnancy was 10.0% in 2000, the prevalence of smokers in this study was small (3.4%). Therefore, the interpretation of the findings may require some consideration. It was possible that the association of neonatal neurobehavioral status with maternal smoking was not examined sufficiently given the small number of smokers. The reason for this small number of smoking mothers is not known, but there are several possibilities. First, it is possible that the smokers were originally few. The present study was done at big hospitals in an urban area. Women who were interested in health participated in our cohort study. Moreover, guidance for smoking cessation is provided pregnant women in the hospitals where this study was conducted. Second, the data on smoking habits were collected by a self-report questionnaire. Objective measurements such as salivary, serum, or urine cotinine levels were not available. Although several studies have yielded modest correlations between self-reported smoking and biomarkers of cigarette exposure (Bennie, Mchugh, Macpherson, Borland, & Moir Malik, 2004; Fendrich, Mackesy-Amiti, Johnson, Hubbell, & Wislar, 2005; Yano, 2005), misclassification of the mothers' cigarette use cannot be ruled out. Ernhart, Morrow-Tlucak, Sokol, and Martier (1988) reported that mothers report more drinking in pregnancy retrospectively than when interviewed during pregnancy. It is possible that these smoking mothers did not describe their actual smoking habits because of hospital provided guidance for smoking cessation. However, since this study is a longitudinal cohort study to monitor the growth of these children, re-administering the questionnaire on maternal smoking habits during pregnancy will be done so re-analysis of the classification of the mothers' cigarette use can be done in the near future.

Several studies have linked reductions of motor function, general cognitive function, or psychiatric disturbances such as attention deficit hyperactivity disorder in childhood, to maternal smoking during pregnancy (Fried, *et al.*, 1998; Trasti, Vik, Jacobsen, & Bakketeig, 1999; Day, Richardson, Goldschmidt, & Cornelius, 2000). This present study is a prospective cohort study, so evaluation of the long-term effects of maternal smoking during pregnancy on child development from diverse angles will be continuous. The clinical significance of a negative change in muscle tone in newborns is poorly understood, but it may be linked to motor, cognitive, or behavioral problems in later childhood. It is important to investigate the association between infant muscle tone status and cognitive or behavioral outcomes during childhood.

Finally, there was an association of a lower score for muscle tone with maternal smoking, but interpretation may require some considerations, given the small number of smokers which may be a limitation of this study. Since this is the first report in which an association between maternal smoking and neurobehavioral status in Japanese infants has been noted, continued examination of the association of maternal smoking during child development from diverse angles is important. Outcomes of this study might be important in considering the effects of smoking by pregnant women in Japan.

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Fish consumption and time to pregnancy in Japanese women

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Abstract

The work described in this paper examined the possible relationship between fish consumption and human fecundity among Japanese women using hair mercury level and time to pregnancy (TTP) as indicators of fish consumption and fecundity. We hypothesized that hair mercury concentrations reflect the level of fish consumption and, consequently, can also be taken to indicate the level of intake of organochlorine compounds (OCs) such as dioxins and polychlorinated biphenyls (PCBs) for which fish are the primary source, and which can disrupt normal human reproductive processes. TTP was obtained by a self-administered questionnaire from women who had been delivered of a baby at either of two hospitals in Sendai, Japan, during the period of January 2002–March 2004. Total mercury concentration in their hair (0–3 cm from the scalp) was determined by cold vapor atomic absorption spectrometry.

Of the 298 women approached, 193 (65%) reported their TTP. The subjects were classified into two groups according to their TTP: group 1, 0–12 months TTP; and Group 2, >12 months TTP. A step-down procedure backward binominal logistic regression analysis was performed by using age, BMI, parity, frequency of intercourse, life-style parameters (smoking, drinking and dietary habits) and hair mercury level of the female subjects and their partners as independent variables. Two separate analyses were performed by including/excluding information on the partners of the subjects.

The analyses did not extract hair mercury concentration as significant indicating that fish consumption did not prolong TTP, which was not consistent with the results of our previous study, i.e., in that study frequent fish eaters showed prolonged TTP. Possible reasons of the negative result are discussed and it is suggested that the hair mercury levels in the present study were not an appropriate indicator of fish consumption of the subjects or of their exposure to OCs. Further study on the relationship between fish consumption (and, ultimately, of OCs intake) and fecundity with more appropriate indicators of fish consumption and/or fish-mediated pollutants intake are warranted to characterize the health risk posed by fish consumption.

Keywords: Time to pregnancy; Fecundity; Hair mercury concentration; Fish consumption; Japanese women

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Introduction

Fish has been the major source of protein for Japanese people since ancient times and its health-giving properties have recently been highlighted by the health authorities in many countries. Japanese people consumed, on average, 94 g of fish and shellfish per person daily in 2001 (Ministry of Health, Labor and Welfare, Japan, 2003). It is important, however, to evaluate the health risks posed by fish consumption because it is well known that fish can be a major source of hazardous chemicals such as polychlorinated biphenyls (PCBs), dioxins, and methylmercury.

We are interested in the possible effects of exposure to environmental chemicals on human fecundity. Time to pregnancy (TTP) has attracted interest in several countries as a measure of human fecundity (Juul et al., 1999). However, until recently it has not been used in reproductive health research in Japan. In our previous study on the applicability of TTP to Japanese subjects, we found a statistical association between frequent fish eating and prolonged TTP (Arakawa et al., 2003). We hypothesized that the prolonged TTP was due to excessive exposure (through fish consumption) to chemicals, particularly to organochlorine compounds (OCs) that can disrupt normal human reproductive processes. Our previous results were consistent with those of Buck et al. (2000), but not with the results of several other studies (Buck et al., 1997, 1999; Axmon et al., 2002). The inconsistency may have resulted from the semi-quantitative estimation of fish consumption, such as fish eating frequency, used in those studies. Moreover, in our previous study, the number of subjects was limited ($n = 92$). It was necessary, therefore, to re-evaluate our findings using a larger population and with a more quantitative indicator of fish consumption.

As a quantitative indicator of fish consumption by subjects from a fish-eating population without occupational mercury exposure, the total mercury concentration in hair has been used (Yamaguchi et al., 1971; Iwasaki et al., 2003). In the work reported here, we investigated the possible relationship between fish consumption and human fecundity in Japanese women using mercury concentration in hair as a quantitative indicator of fish consumption.

Materials and methods

Study design and sampling

We approached the subjects in a prospective cohort on child development and environmental exposure to chemicals (Nakai et al., 2004). Women who had been delivered of a baby at either of two hospitals in Sendai, Japan during the period of January 2002–March 2004, were asked to participate in our study. Only women who had given written consent after having the purpose and design of our study explained to them were included in the investigation.

Hair samples were obtained from the subjects on the second day postpartum. A lock of hair was cut with stainless steel scissors as close to the scalp as possible. Samples were placed in plastic bags such that the scalp end of the hair was identifiable in each case and they were kept in desiccators until analysis.

On the third day postpartum subjects were asked to complete questionnaires that had been developed in our preliminary study (Arakawa et al., 2003). The questionnaire included questions on biological attributes and life style parameters (dietary habits, smoking, drinking, the intake of caffeinated beverages, etc.) of both the woman before pregnancy and of her partner, and on TTP (How long did you try to become pregnant after cessation of contraception?). Dietary habits were revealed by questions about the frequency of consumption per week, and beverage consumption by the number of cups per week.

The Ethical Committee of Tohoku University approved this study.

Analytical methods

Clippings from the scalp end of the lock of hair, 3 cm in length, were taken and subjected to mercury analysis. Determination of total mercury concentration was carried out by cold vapor atomic absorption spectrometry (Akagi and Nishimura, 1991) with minor modifications. Analytical accuracy was ensured by analyzing the Human Hair Reference Material NIES CRM No. 13 from the National Institute of Environmental Studies (Lot #650, Tsukuba, Japan). Details of the mercury analysis are given elsewhere (Nakai et al., 2004).

Statistical methods

The subjects were classified into two groups according to the reported TTP: group 1 (TTP 0 – 12 months) and group 2 (TTP >12 months). Biological attributes and life-style parameters of both the subject and her partner were compared between the two groups by chi-square tests and by t-tests. The body mass indices (BMI) of the subjects were calculated from the reported weights and heights before pregnancy. Caffeine consumption was calculated from the cups-per-week-consumption and the caffeine contents of the beverages (Bunker and McWilliams, 1979, Wilcox et al., 1988, Stanton and Gray, 1995). Correlations between fish consumption and total mercury concentrations in hair were analyzed by the

Spearman rank correlation analysis. A step-down procedure backward binominal logistic regression analysis was employed to extract statistically significant independent variable(s) that contributed to classify the subjects into groups 1 and 2. Independent variables used in these analyses were biological attributes (age, parity, and BMI), frequency of intercourse, total mercury concentration in hair, and life style parameters (smoking, alcohol intake, caffeine intake, frequencies of milk and meat consumption) of both the female subject and her partner. Since there was a significantly positive correlation between the subject and her partner for age and some of the life-style parameters, it was not considered appropriate to include both of these variables for the subject and her partner as independent variables. Therefore, two separate binominal logistic regression analyses were carried out: one by using age and life-style parameters of the female subject only, and another by combining those variables for the subject and her partner (e.g., sum of the ages of the subject and her partner). Cox regression was also performed using the same variables as those of the logistic regression analysis, but TTP as continuous variable. Statistical analysis was performed using SPSS for Windows version 11.5.

Results

Of the 298 women approached, 193 (65%) reported their TTP. We excluded subjects who got pregnant in spite of contraception and who had had infertility treatment from the data analysis (four and nine women, respectively) because their TTP may not have represented actual fecundity. Thus, 180 women were eligible for statistical analysis. Fig. 1 shows the TTP distribution of the 180 subjects. Comparison of demographic parameters for subjects who reported TTP and those who did not was given in Table 1. This table showed that there were significant differences in some parameters between the two groups.

The geometric mean of the total mercury concentrations in hair was 2.01 μg/g (range 0.42–9.35 μg/g, $n = 177$). There was a significant association between frequency of fish consumption and total mercury concentrations in hair ($r = 0.19$, $p = 0.01$, Fig. 2).

Table 2 compares biological attributes and life style parameters of both the subject and her partner for the two groups defined according to TTP. Meat consumption of partner was the only variable that was significantly different between the groups. Approximately, 60% of the subjects and >80% of their partners consumed fish more than 3 meals/week for total subjects (Table 1) and for those reported TTP (Table 2). Fig. 3 shows total mercury concentration in the hair of subjects divided by reported TTP. The geometric means of mercury concentrations in hair were 2.01 μg/g for group 1 and 1.97 μg/g for group 2, and the difference was not statistically significant.

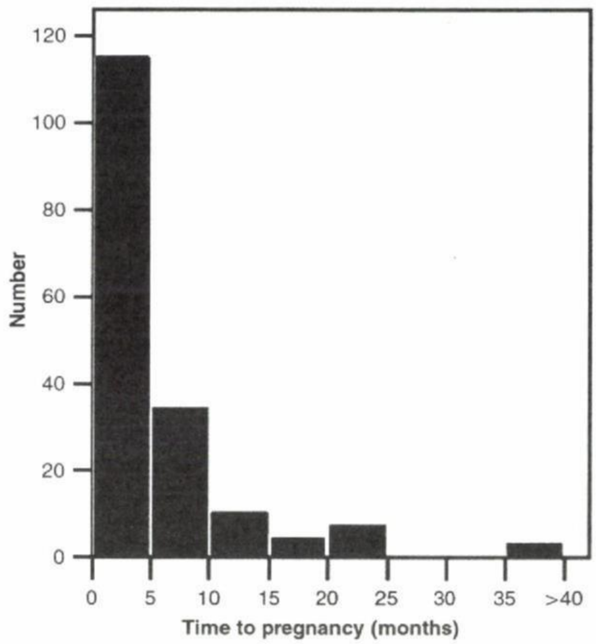

Fig. 1. Distribution of time to pregnancy ($n = 180$).

Alcohol consumption was the only variable that was extracted for prolonged TTP by a step-down procedure backward binominal regression analysis when age and lifestyle parameters of the female subject only were included (Table 3). When summed variables were used, prolonged TTP was associated with decreasing parity and increasing sum of age of the subject and her partner (Table 4). Sum of age and parity were the variables with marginal insignificance ($p = 0.06$) found in Cox regression analysis for the summed variables. Total mercury concentration in hair was not selected as significant in either of the analyses. When hair mercury concentration was replaced with frequency of fish consumption in the logistic regression analyses, the frequency was not selected as significant either.

Discussion

The response rate to the question of TTP asked of the present subjects (65%) was slightly lower than that of our previous study (75% response rate). This may have resulted from the fact that information on TTP was sought at 2–3 gestational months in our previous study and at postpartum in this study. However, the distribution of the reported TTP for our subjects (Fig. 1) was