研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Arakawa C, Satoh H. et al.	Fish consumption and time to pregnancy in Japanese women.	Int J Hyg Environ Health	9	337-344	2006
Sakamoto M, Satoh H. et al.	Correlations between mercury concentrations in umbilical cord tissue and other biomarkers of fetal exposure to methylmercury in the Japanese population.	Environ Res.	1	106-111	2007
Murata K, Satoh H.et al.	Subclinical effects of prenatal methylmercury exposure on cardiac autonomic function in Japanese children.	Int Arch Occup Environ Health.	79	379-386	2006
Suzuki K, Satoh H. et al.	Association of maternal smoking during pregnancy and infant neurobehavioral status.	Psychol Rep.	99	97-106	2006
Kuriyama S, Tsubono Y. et al.	Green tea and the risk of colorectal cancer: pooled analysis of two prospective studies in Japan.	JAMA	296	1255-1265	2006
Ishikawa A, Tsubono Y. et al.	Smoking, alcohol drinking, green tea consumption and the risk of esophageal cancer in Japanese men.	J Epidemiol.	16	185-192	2006
Kikuchi N, Tsubono Y. et al.	No association between green tea and prostate cancer risk in Japanese men: the Ohsaki Cohort Study.	Br J Cancer.	95	371-373	2006

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Sato Y, Tsubono Y. et al	Meat consumption and risk of colorectal cancer in Japan: the Miyagi Cohort Study.	Eur J Cancer Prev..	15	211-218	2006
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Sato N., Yaegashi N. et al.	Expression of the Organic Cation Transporter SLC22A16 in Human Endometrium	Int J Gynecol Pathology	26	53-60	2007
Hayashi S., Yaegashi N. et al.	Biosynthesis and action of estrogen in gynecological cancers.	Reproductive Oncology		In press	2007
Yoshinaga K, Yaegashi N. et al.	Phase I trial of concurrent chemoradiation with weekly nedaplatin in patients with squamous cell carcinoma of the uterine cervix.	Gynecol Oncol.	104	36-40	2007
Okamura C., Yaegashi N. et al.	Lactation and risk of endometrial cancer in Japan: A case-control study.	Tohoku Journal of Experimental Medicine	208	109-115	2006

Ⅴ. 研究成果の刊行物・別刷

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Correlations between mercury concentrations in umbilical cord tissue and other biomarkers of fetal exposure to methylmercury in the Japanese population

Mineshi Sakamoto[a,*], Tsuyoshi Kaneoka[b], Katsuyuki Murata[c], Kunihiko Nakai[d], Hiroshi Satoh[d], Hirokatsu Akagi[e]

[a]*Department of Epidemiology, National Institute for Minamata Disease, Kumamoto 867-0008, Japan*
[b]*Department of Obstetrics and Gynecology, Fukuoka University Graduate School of Medicine, Fukuoka 814-0180, Japan*
[c]*Department of Environmental Health Sciences, Akita University School of Medicine, Akita 010-8543, Japan*
[d]*Department of Environmental Health Sciences, Tohoku University Graduate School of Medicine, Miyagi 980-8575, Japan*
[e]*International Mercury Laboratory Inc., Kumamoto 867-0034, Japan*

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Abstract

Methylmercury (MeHg) is one of the most risky substances to affect humans through fish consumption, and the fetus is known to be in the most susceptible group. Our objective in this study is to examine the relationships of total mercury (THg) and MeHg concentrations between umbilical cord tissue and other tissues as biomarkers of fetal exposure to MeHg in the Japanese population. In total, 116 paired samples were collected in three Japanese districts, the Tsushima Islands, Fukuoka City, and Katsushika ward of metropolitan Tokyo. THg was measured for hair and THg and MeHg were measured in cord tissues, maternal blood, and cord blood. The relationships among tissues in Hg concentrations were similar among districts. Therefore, we analyzed the relationships using all the samples. More than 90% of Hg in cord tissue, cord blood, and maternal blood was MeHg. THg and MeHg in cord blood was about two times higher than in maternal blood. A strong correlation was found between THg and MeHg in cord tissue. The cord tissue THg and MeHg showed a strong correlation with cord blood Hg, which is recognized as the best biomarker for fetal exposure to MeHg. The findings of this study indicate the significance of cord tissue THg and MeHg as biomarkers for fetal exposure to MeHg at parturition.

Keywords: Mercury; Methylmercury; Exposure; Umbilical cord tissue; Umbilical cord blood; Maternal blood; Hair; Parturition; Biomarkers; Fetus; Parturition

1. Introduction

Fetuses are known to be a high-risk group for methylmercury (MeHg) exposure because of the high susceptibility of the developing brain itself (Choi, 1989; Sakamoto et al., 1993; World Health Organization (WHO), 1990). Moreover, MeHg easily crosses the blood–placenta barrier, accumulating more in the fetus than the mother (Choi, 1989; Sakamoto et al., 2002, 2004; Stern and Smith, 2003; WHO, 1990). Therefore, the effect of MeHg exposure on pregnant women is an important issue for elucidation, especially in Japanese and some other populations that consume much fish and sea mammals (Grandjean et al., 1997, 2005; Myers et al., 1995a, b, 2003; National Research Council (NRC), 2000; WHO, 1990).

The epidemic called “Minamata disease” is well known as the first instance on record of severe MeHg poisoning caused by manmade environmental pollution, which occurred mainly among fishermen and their families in and around Minamata City. It originated from the consumption of large amounts of fish and shellfish contaminated with MeHg discharged from a chemical plant (Irukayama and Kondo, 1966). The principal

*Corresponding author. Fax: +81 966 61 1145.
E-mail address: sakamoto@nimd.go.jp (M. Sakamoto).

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symptoms included neurological disorders such as sensory disorders, cerebellar ataxia, contraction of the visual field, hearing impairments, and disequilibrium (Takeuchi et al., 1962). The first patient was reported in 1953, and the number of patients rapidly increased after 1955. Up to the present, more than 2000 people have been certified to have Minamata disease. Furthermore, many fetuses exposed to MeHg through the placenta of the exposed mother showed severe cerebral-palsy-like symptoms, while their mothers had mild or no manifestation of the poisoning (Harada, 1978). Outbreaks of the typical fetal-type Minamata disease occurred during 1955–1959 when the mercury pollution appears to have been most severe, judging from the incidence of patients (Harada, 1978) and the MeHg concentration in the preserved umbilical cords of inhabitants of the area (Nishigaki and Harada, 1975). During the period, a deceased male birth ratio associated with increased male fetal death was observed in Minamata City pollution, indicating its severity and widespread distribution (Sakamoto et al., 2001). This landmark epidemic was the first to bring worldwide attention to the high risk of fetal exposure to MeHg.

Thereafter, large prospective cohort studies were conducted in the Seychelles (Myers et al., 1995a, b, 2003) and the Faroe Islands (Grandjean et al., 1997, 1999, 2005), where fish or sea mammal consumption is high. Though the MeHg exposure level was similar in these studies, different conclusions were reached (NRC, 2000). The exposure level in the epidemic in Minamata is thought to have been much higher than in those studies. Therefore, the study of Minamata disease is still very important, and it will provide more obvious health effect information by using recently developed neurological test batteries. Fortunately, Japanese people have a custom of preserving a small piece of the dried umbilical cord tissue as a birth memento in a wooden or plastic box deep inside a chest of drawers. MeHg in the tissue will not be reduced by microorganisms, because it is completely dried. By measuring the Hg concentrations in this preserved dry cord sample, we can estimate the individual MeHg exposure level at birth. Grandjean et al. (2005) revealed that cord tissue Hg as well as cord blood Hg was useful as a predictor of the effect of fetal exposure to MeHg. However, the preserved cord tissues had often been treated with mercurochrome at the period when cut off at parturition. The mercurochrome can easily dissociate into Hg ions in the solution and the ions act as a disinfectant. Then the cord tissue treated with mercurochrome shows a very high Hg concentration when total mercury (THg) is measured. Accordingly, we need to measure not THg but MeHg in the preserved cord tissue in Japan, especially in the samples treated back in those days. Some studies (Akagi et al., 1998; Nishigaki and Harada, 1975) have revealed exposure of the fetus to MeHg in the Minamata area by measuring it in the preserved cord tissue. The present study investigates the relationships between THg and MeHg in cord tissue and the relationships between other biomarkers of fetal exposure to MeHg in the Japanese population to evaluate the significance of the Hg concentrations in cord tissue.

2. Materials and methods

2.1. Subjects and sampling

In total, 116 healthy Japanese pregnant women without any special exposure to mercury, ranging in age from 19 to 41 yr (average 30.0 ± 5.0), gave informed consent to take part in the present trial. The samples were collected in the Tsushima Islands in Nagasaki Prefecture (30 cases), Fukuoka City in Fukuoka Prefecture (68 cases) and Katsushika, a special ward of metropolitan Tokyo (18 cases). Blood samples from the mothers and umbilical cord were collected immediately after birth in 1996. Whole length of maternal hair and cord tissue near the fetus were also collected at parturition. Samples were stored at −80 °C until analysis. About 1 cm of umbilical cord from the fetus side was rinsed in physiological saline solution to remove blood and body fluid and pressed between paper towels to remove the saline solution. Further, they were dried at 40 °C for 3 days and were kept in desiccators. No further weight loss occurred after the dehydration, ensured by weighing for 3 days. The dried tissues were then cut into small pieces with scissors and around 0.1–0.2 g of the tissues were moistened with 0.5 ml of water one day prior to the Hg analysis. This study was approved by the Ethics Committee of the National Institute for Minamata Disease (NIMD).

2.2. Mercury analysis

THg in the samples was determined by cold vapor atomic absorption spectrophotometry (CVAAS) according to the method of Akagi et al. (2000). The method involves sample digestion with HNO_3, $HClO_4$, and H_2SO_4 (1 + 1 + 5), followed by reduction to elemental Hg vapor by $SnCl_2$. The detection limit was 0.01 ng/g. MeHg in the samples was determined by gas chromatography with electron capture detection (GC-ECD) according to the method of Akagi et al. (2000). The method involves sample digestion with KOH–ethanol and subsequently, under slightly acidic conditions, the fatty content is removed using *n*-hexane. After extraction with dithizone–toluene, MeHg is back-extracted with a slightly alkaline sodium sulfide solution. The excess sulfide ions are then removed as hydrogen sulfide by purging with nitrogen gas after slight acidification with HCl solution. MeHg is then re-extracted with a small portion of dithizone–toluene; the extract is washed with NaOH solution to remove the excess dithizone, and then slightly acidified with HCl and analyzed by GC-ECD. The detection limit was 0.01 ng/g. Accuracy of THg was ensured by using reference blood material Level 2, MR9067 (Seronorm201605: Nycomed Co., Oslo, Norway): the THg determined averaged 7.5 μg/L, as compared to the range of 6.8–8.5 μg/L recommended by ICP-SFMS. The precision of the method, expressed as coefficient of variation, was 0.8%. Analysis of a MeHg standard solution containing 5 μg/L gave a recovery of almost 100%. The precision and accuracy of THg and MeHg were repeatedly verified by interlaboratory calibration exercises, including the analysis of standard reference material such as IAEA-085, 086, and 142 (Horvat et al., 1988).

2.3. Statistics

THg and MeHg concentrations among the districts were analyzed by one-way analysis of variance (ANOVA). Similar correlations were observed between the Hg concentrations in each tissue among the districts. Therefore, all the data were combined and the associations between THg and MeHg among samples were studied by Pearson correlation analysis. Logarithmic transformation was used to correct the skewed distribution of the Hg concentrations. The differences in Hg concentrations between paired samples were determined by paired *t*-test. A *P*-value less than or equal to 0.05 was considered to demonstrate statistical significance.

Table 1
Geometric means and 25th–75th percentiles of total mercury (THg) in hair and total and methylmercury (MeHg) in cord tissue, maternal blood and cord blood in three districts in Japan

Districts (ng/g)	Hair	Cord tissue		Maternal blood		Cord blood	
	THg	THg	MeHg	THg	MeHg	THg	MeHg
Tsushima ($n = 30$)	1453 (1157–1950)	64.0 (46.7–87.9)	54.6 (38.1–71.8)	4.62 (3.02–7.55)	3.98 (2.75–5.95)	9.13 (6.37–12.4)	8.38 (5.84–10.7)
Fukuoka ($n = 68$)	1954 (1170–2293)	97.0 (80.6–125)	89.3 (75.8–121)	4.9 (3.65–6.44)	4.61 (3.61–5.73)	9.27 (7.03–13.9)	8.93 (6.57–11.5)
Katsushika ($n = 17$)	2120 (1810–2990)	137.7 (94.5–207)	130.8 (95.5–197)	7.91 (5.42–11.3)	7.54 (5.21–10.3)	13.9 (8.87–20.9)	11.4 (8.13–20.1)
Total ($n = 115$)	1624 (1175–2195)	91.7 (63.5–127)	83.1 (57.1–122)	5.18(3.63–7.34)	4.77 (3.5–6.54)	9.81 (6.96–13.6)	9.32 (6.56–13.4)
MeHg (%) Mean±SD			90.6±10.4		92.5±8.1		95.2±5.5

Note: The mean MeHg percentage (MeHg/THg × 100).

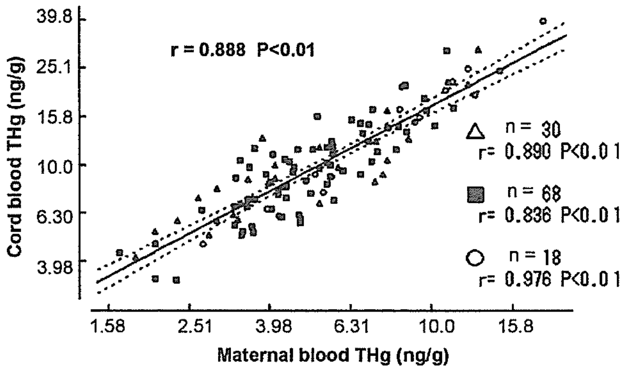

Fig. 1. Correlation between total mercury (THg) in maternal and cord blood: (△) Tsushima Islands; (■) Fukuoka City; and (○) Katsushika ward of metropolitan Tokyo. Dotted lines represent 95% confidence intervals for the regression line.

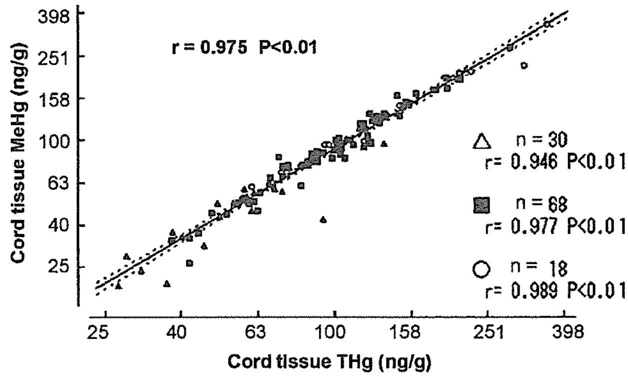

Fig. 2. Correlation between total mercury (THg) and methylmercury (MeHg) in cord tissue: (△) Tsushima Islands; (■) Fukuoka City; and (○) Katsushika ward of metropolitan Tokyo. Dotted lines represent 95% confidence intervals for the regression line.

3. Results

Table 1 presents the geometric means of THg in hair and THg and MeHg in cord tissue, maternal blood, and cord blood in three districts in Japan. There were significant differences among the districts in all the Hg concentrations ($P<0.01$). The geometric means of Hg concentrations in all tissues were highest in Katsushika ward of metropolitan Tokyo, followed by Fukuoka City and the Tsushima Islands. All the data were combined, because similar correlations were observed among the Hg concentrations in all tissues among the districts as shown in Figs. 1–3. Significant correlations of THg and MeHg concentrations were observed among the biomarkers as shown in Table 2. THg and MeHg in cord tissue showed strong correlations with those in maternal and cord bloods (Table 2). However, the correlation coefficients of THg in maternal hair and THg and MeHg in other biomarkers were comparatively low and scattered, as shown in Table 2 and Fig. 3. As shown in Table 2 and Fig. 1, the geometric means of THg and MeHg in cord blood were 9.81 and 9.32 ng/g, respectively, and the concentrations were about two times higher ($P<0.01$) than those of maternal blood (5.18 and 4.77 ng/g). THg and MeHg in cord tissue showed

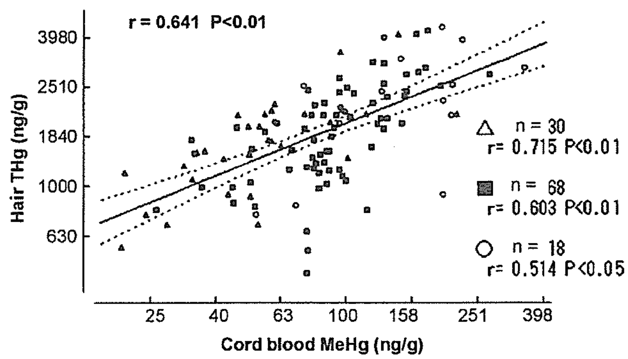

Fig. 3. Correlation between cord tissue methylmercury (MeHg) and hair total mercury (THg): (△) Tsushima Islands; (■) Fukuoka City; and (○) Katsushika ward of metropolitan Tokyo. Dotted lines represent 95% confidence intervals for the regression line.

strong correlation coefficients (Table 2 and Fig. 2), and the geometric means of THg and MeHg in cord tissue were 91.7 and 83.1 ng/g, respectively. The mean MeHg percentage (MeHg/THg × 100) in cord tissue was 90.6±10.4%. MeHg percentages in cord blood and maternal blood were 95.2% and 92.5%, respectively (Table 1), and the mean MeHg percentage in cord blood was significantly ($P<0.01$) higher than that of maternal blood.

Table 2
Correlation coefficients of logarithmic total mercury (THg) in hair and total and methylmercury (MeHg) in cord tissue, maternal blood and cord blood in the total of three districts in Japan

	Hair	Cord tissue		Maternal blood		Cord blood	
	THg	THg	MeHg	THg	MeHg	THg	MeHg
Hair THg	1						
Cord tissue THg	0.641	1					
Cord tissue MeHg	0.626	0.975	1				
Maternal blood THg	0.648	0.809	0.799	1			
Maternal blood MeHg	0.654	0.846	0.839	0.981	1		
Cord blood THg	0.651	0.848	0.815	0.888	0.885	1	
Cord blood MeHg	0.646	0.873	0.839	0.882	0.888	0.992	1

Note: All the correlation coefficients were significant ($P<0.01$).

4. Discussion

MeHg is one of the most risky substances for the fetal brain, and most of the human exposure to MeHg is through maternal fish consumption. MeHg easily passes through the placenta as a cysteine conjugate during intrauterine life (Aschner and Clarkson, 1987; Kajiwara et al., 1996). The National Research Council (NRC, 2000) recommended cord blood Hg as the best biomarker for fetal exposure to MeHg. In addition, cord tissue Hg concentration was revealed to be useful as a predictor of the effect of fetal exposure to MeHg (Grandjean et al., 2005). The MeHg concentration in preserved umbilical cord was also used as a biomarker of fetal-type Minamata disease patients (Akagi et al., 1998; Nishigaki and Harada, 1975). The present study investigated the relationships between THg and MeHg in cord tissue and other biomarkers of fetal exposure to MeHg in the Japanese population to evaluate the significance of Hg in the tissue.

The differences in MeHg exposure levels in various geographic areas were similar to the data from a recent Japanese hair mercury survey (Yasutake et al., 2004). The high Hg concentrations in katushika ward of Metropolitan Tokyo may also be explained by the high amount of tuna consumption in Tokyo and nearby Tokyo Metropolitan Prefecture (Yasutake et al., 2004). The correlations between the Hg concentrations in biomarkers were similar among areas. The geometric mean of THg in cord tissue in this study was 91.7 ng/g, and the level was approximately half that of the Faroe Islands study (Dalgard et al., 1994; Grandjean et al., 2005).

Thanks to the traditional Japanese custom of preserving umbilical cord tissue at the time of the infant's birth, we may use this dried cord tissue to estimate past MeHg exposure. The cord tissue is formed mainly during the second and third trimester, and cord blood is the blood circulating in the fetal body at parturition. Judging from the data on the biological half-life of MeHg of about 45 days (Smith and Farris, 1996), not only fetal blood but also cord tissue Hg will reveal the average MeHg burden of the fetus during the third trimester. In addition, rapid brain growth occurs primarily during the third trimester in humans (Dobbing and Sands, 1979) and the brain at the period is known to be most vulnerable to the toxicity of MeHg (Rice and Barone, 2000). Strong correlation coefficients were observed between cord blood THg, which is recommended as the best biomarker for fetal exposure to MeHg by the National Research Council (NRC, 2000). The strong correlation coefficient between THg and MeHg in cord tissue and the high MeHg percentage (about 90%) also suggest that cord tissue MeHg as well as THg concentrations are useful biomarkers for prenatal MeHg exposure.

Under the steady-state condition, the hair/blood mercury ratio is about 250 (WHO, 1990). However, in the present study, the maternal hair/maternal blood ratio was about 350, presumably due to the lower hematocrit (Htc, the ratio of the volume of red blood cells to the total volume of blood) during gestation (Bollini et al., 2005), especially in the last trimester as plasma volume increases. The low maternal Htc during gestation will explain the higher hair/blood ratio, because about 90% of Hg exists in red blood cells in a population that consumes much fish (Group, 1970; Sakamoto et al., 2002; Svensson et al., 1992). The difference in MeHg% between maternal blood (92.5%) and cord blood (95.2%) may also be explained by the low Htc in the former blood and the high Htc in the latter blood, as indicated by Sakamoto et al. (2002).

Maternal hair and maternal blood Hg concentrations are also important biomarkers for fetal MeHg exposure. Originally, maternal biomarkers reflect the exposure of the mother herself, and there is a certain variability between the maternal and fetal MeHg levels. Our recent study indicated that individual cord/maternal Hg concentrations in red blood cells varied from 1.08 to 2.19 in mother–infant 53 pairs at parturition, which show the individual differences in MeHg concentrations between maternal and fetal circulations at late gestation (Sakamoto et al., 2004). Stern and Smith (2003) also summarized the variability of cord/maternal blood Hg level ratio. Grandjean et al. (1997, 2005) revealed significant associations between the adverse effects and the Hg level in cord blood and cord tissue, but the effects were not well associated with the level in maternal hair. In the present study, THg and MeHg in cord showed strong correlations with those in maternal and cord blood. However, the correlations of THg in maternal hair and either THg or MeHg in other biomarkers were comparatively low, and the 95% confidence interval of the intercept for the regression line did not include zero. This may have been due to the fact that we used the whole length of hair for Hg analysis in the present study, while Hg levels in newly formed hair reflect those in blood (Phelps et al., 1980) . In this way, the Hg concentrations in whole hair do not exactly reflect the Hg level in blood at parturition. In addition, another reason for the scattered distribution would be decrease in Hg level by artificial hair waving (Dakeishi et al., 2005; Yamamoto and Suzuki, 1978).

Some of the ratios among biomarkers calculated from our study were similar to those calculated from the Faroe Island study (Grandjean et al., 1997, 2005), suggesting that the ratios are applicable to estimation of the past Hg levels in other tissues at parturition in a population in which the MeHg exposure level is comparatively high. The mean cord tissue Hg level of fetal-type Minamata disease patients ($n = 24$, median MeHg = 1.63 μg/g; Akagi et al., 1998) was about eight times higher than that in the Faroe Islands ($n = 447$, geometric mean THg = 0.21 μg/g; Grandjean et al., 1997, 1999), and about 20 times higher than our present result (geometric mean MeHg = 0.083 ng/g). The estimated mean THg in maternal blood, cord blood, and maternal hair of the fetal-type of Minamata disease patients were approximately 100, 200 ng/g and 32 μg/g, respectively. However, the estimation of the maternal hair THg will be more uncertain as we mentioned earlier.

The findings of this study support the use of umbilical cord THg and/or MeHg as biomarkers of fetal exposure to MeHg. Further, the MeHg concentration in preserved cord tissue will be useful as the only biomarker available as a predictor of the retrospective dose–response (or dose–effective) study in the Minamata district even up to today.

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This study was approved by the Ethics Committee of the National Institute for Minamata Disease (No. 19-55-4 NIMD).

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Fish consumption and time to pregnancy in Japanese women

Chikako Arakawa[a], Jun Yoshinaga[a,*], Kunihiro Okamura[b], Kunihiko Nakai[c], Hiroshi Satoh[c]

[a]*Institute for Environmental Studies, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*
[b]*Department of Obstetrics, Graduate School of Medicine, Tohoku University, Japan*
[c]*Department of Environmental Health Sciences, Graduate School of Medicine, Tohoku University, Japan*

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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

*Corresponding author. Present address: Kashiwanoha 5-1-5, Kashiwa, Chiba 277-8563, Japan. Tel./fax: 81 4 7136 4716.
E-mail address: junyosh@k.u-tokyo.ac.jp (J. Yoshinaga).

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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.

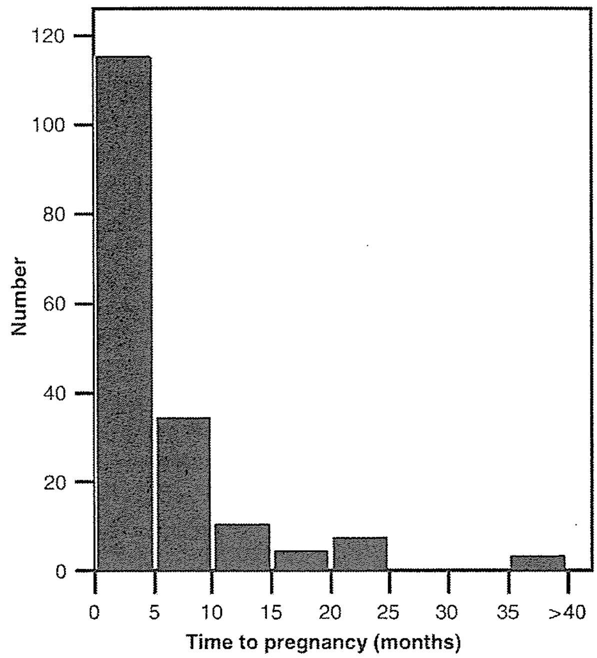

Fig. 1. Distribution of time to pregnancy ($n = 180$).

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.

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

Table 1. Comparison of characteristics of the participants who reported/did not report their TTP

		TTP		Total	*p* value
		Not reported	Reported		
Number		118	180	298	
Age (years)					
Subject		31.9	30.6	31.4	0.14
Partner		33.7	31.9	32.9	0.05
BMI (kg/m^2)		21.2	20.6	20.8	0.08
Number of births (times)		1.9	1.5	1.7	<0.001
Hair mercury level (μg/g)		2.02	2.01	2.01	0.94
Frequency of intercourse (%)					
<1/week		51	41	44	0.19
1/week		30	40	37	
>2/week		19	19	19	
Smoking (%)					
Subject		25	16	19	0.05
Partner		62	54	57	0.20
Milk (glass/week)					
Subject		4.4	5.3	4.9	0.09
Partner		2.6	4.4	3.7	<0.001
Caffeine (mg/week)					
Subject		1114	1332	1247	0.04
Partner		1188	1337	1280	0.17
Alcohol (g/week)					
Subject		75.7	69.7	72.0	0.75
Partner		183	170	175	0.77
Fish consumption (%)					
Subject	<1/week	2.9	1.7	2.1	0.65
	1–2/week	41	39	40	
	3–5/week	42	49	47	
	>5/week	14	11	12	
Partner	<1/week	2.9	1.7	2.1	0.34
	1–2/week	21	17	19	
	3–5/week	57	62	60	
	>5/week	19	19	19	
Meat consumption (%)					
Subject	<1/week	3.9	4.0	3.9	0.98
	1–2/week	38	36	37	
	3–5/week	44	46	46	
	>5/week	14	14	14	
Partner	<1/week	1.0	0.0	0.4	0.40
	1–2/week	20	15	17	
	3–5/week	54	55	55	
	>5/week	25	30	28	

similar to those reported previously (e.g., Curtis et al., 1999) as well as to the distribution obtained in our previous study on Japanese subjects (Arakawa et al., 2003). The present result again shows that the TTP questionnaire is applicable to Japanese women in spite of possible differences in their sexual practices compared to those of Western women, e.g., in their preferred methods of contraception.

The geometric mean of the total mercury concentrations (2.01 μg/g) was close to those obtained in a recent

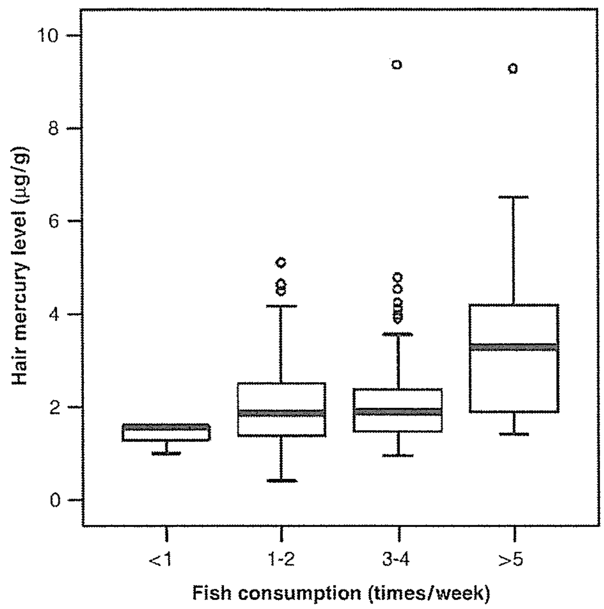

Fig. 2. Relationship between frequency of fish consumption (meals per week) and hair mercury level.

study in which geometric mean values of 1.23–2.50 μg/g were reported for mercury concentrations in the hair of persons from 5 cities in Japan (total number of samples 1666) (Yasutake et al., 2003). This result indicated that fish consumption of our subjects was similar to that of the average Japanese.

There were significant differences in some parameters between the subjects who reported TTP and those who did not (Table 1). Subjects who did not report TTP were older and had a higher number of previous births. The difference in parity could be explained by the age difference: there was a positive correlation between age and parity for the subjects ($r = 0.33$, $p < 0.001$), and the slope of the regression line indicated that the differences in parity were consistent with age difference. Older women who already have children probably do not memorize TTP because they are likely to pay less attention to getting pregnant. In other words, women who were more anxious about and paid more attention to getting pregnant were the subjects of our analysis. Whether this bias has a bearing on our research objectives should be carefully considered.

In our multivariate analyses, parity and age were significant variables that contributed to prolonged TTP when partners' effects were taken into consideration (Table 4). This result was consistent with those of other studies that reported associations between parity and TTP (e.g., Howe et al., 1985; Olsen, 1991), age of female subject and/or her partner and TTP (e.g., Baird and Wilcox, 1985, Howe et al., 1985; Olsen, 1991; Spinelli et al., 1997). Inconsistent results for the logistic regression analysis were obtained when age and life-style parameters for the subject only were included although the reason for the inconsistency was not clear. The reason why alcohol intake was a significant variable for the classification of our subjects into the two groups (Table 3) was also not clear. The results of logistic regression analysis were virtually the same when Cox regression analysis was employed, though statistical significance was not obtained ($p = 0.06$). However, the important finding was that hair mercury concentration was not selected as significant in either of logistic regression analysis and Cox regression analysis.

It is well known that some OCs, such as dioxins and PCBs, can disrupt normal human endocrine functions (Longnecker et al., 1997; Watanabe et al., 1999), and that these compounds concentrate along food chains (IPCS, 1992; Rolff et al., 1993) and thus fish can contain OCs at high concentrations. Therefore, fish consumption is the predominant route of the intake of OCs for the general population with fish-eating habits (Svensson et al., 1991; Tsutsumi et al., 2001). In addition to this, the total mercury concentration in hair has been considered a quantitative indicator of fish consumption (Yamaguchi et al., 1971; Iwasaki et al., 2003). Thus, we employed hair mercury level as a marker of fish consumption, and of the exposure level to OCs, to relate to TTP, based on the assumption that there is a strong correlation between the concentrations of OCs and mercury in fishes, and consequently, in the tissues and body fluids of humans who consume fish.

The result of this study, however, was not consistent with our previous finding that frequent fish eaters showed prolonged TTP (Arakawa et al., 2003). The reasons for this inconsistency may partly be attributable to the fact that we employed total mercury concentration in the hair segment 0–3 cm from the scalp. The hair mercury level represents fish consumption in the last trimester of the subjects' gestation: clearly this does not correspond to the period of TTP. It is possible that the amount of fish consumption changed after pregnancy. This may be the reason for the low correlation between fish-eating frequency and hair mercury level (Fig. 2), which, though statistically significant, was weaker than those recorded in previous studies (Bjornberg et al., 2005). We should, perhaps, have employed samples of hair of >10 cm from the scalp.

Another possible reason for the negative result of the present study was that hair mercury levels are not correlated with the intake of the chemicals in fish that can affect fecundity. We assumed that the concentrations of OCs and mercury in marine foods would correlate with each other because of their co-biomagnification. However, Bjerregaard and Hansen (2000) reported that the concentrations of OCs and mercury did not correlate in the blood of women who consumed marine food. Therefore, it is clear that we should employ direct indicators that reflect the level of exposure to OCs

Table 2. Comparison of characteristics of the participants between group 1 (TTP: 0–12 months) and group 2 (TTP: >12 months)

		Time to pregnancy		Total	p value
		0–12	>12		
Number		160	20	180	
Age (years)					
Subject		30.6	31.0	30.6	0.70
Partner		31.7	33.7	31.9	0.10
BMI (kg/m^2)		20.7	20.5	20.6	0.83
Number of births (times)		1.5	1.4	1.5	0.23
Hair mercury level (μg/g)		2.01	1.97	2.01	0.82
Frequency of intercourse (%)					
<1/week		40	50	41	0.24
1/week		40	45	40	
>2/week		21	5.0	19	
Smoking (%)					
Subject		15	18	16	0.41
Partner		51	64	54	0.10
Milk (glass/week)					
Subject		5.5	3.4	5.3	0.05
Partner		4.5	3.9	4.4	0.62
Caffeine (mg/week)					
Subject		1314	1469	1332	0.48
Partner		1313	1469	1337	0.30
Alcohol (g/week)					
Subject		59.1	155	69.7	0.12
Partner		169	182	170	0.79
Fish consumption (%)					
Subject	<1/week	1.9	0.0	1.7	0.94
	1–2/week	39	40	39	
	3–5/week	49	50	49	
	>5/week	11	10	11	
Partner	<1/week	1.9	0.0	1.7	0.29
	1–2/week	16	30	17	
	3–5/week	61	65	62	
	>5/week	21	5.0	19	
Meat consumption (%)					
Subject	<1/week	4.5	0.0	4.0	0.68
	1–2/week	36	35	36	
	3–5/week	45	55	46	
	>5/week	15	10	14	
Partner	<1/week	0.0	0.0	0.0	0.03
	1–2/week	14	25	15	
	3–5/week	53	70	55	
	>5/week	33	5.0	30	

to relate to TTP. To date, we know of only one such study: Axmon et al. (2004) investigated the relationship between serum/plasma 2,2′4,4′,5,5′-hexachlorobiphenyl (CB-153) concentrations, a biomarker of total PCB exposure, and TTP. They did not find any association between blood CB-153 concentrations and TTP. They estimated the blood concentrations of CB-153 for the subjects when the index pregnancies took place, namely, 1–36 years previously (median: 20 years), from the CB-153 concentrations in blood that had been drawn from

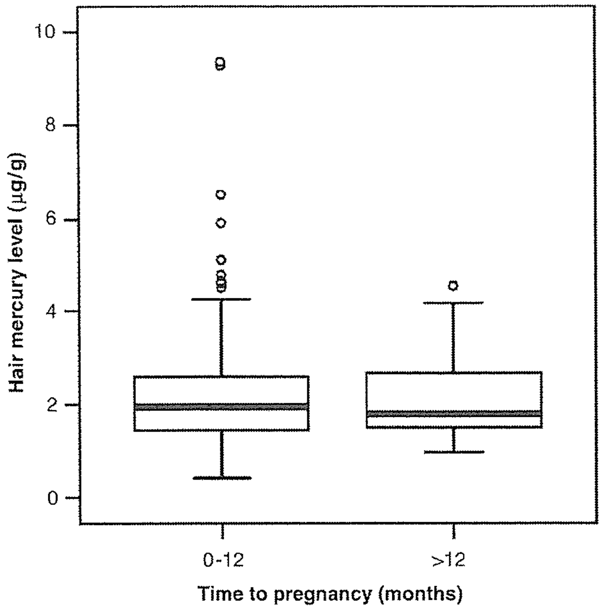

Fig. 3. Comparison of hair mercury level between group 1 (TTP: 0–12 months) and group 2 (TTP > 12 months).

Table 3. Odds ratio of risk factors

	Odds ratio	95% CI	*p* value
Alcohol	1.61	1.05–2.49	0.03

The variable "age" "milk" "alcohol" "caffeine" "meat" "smoking" of the female subjects only were used.

Table 4. Odds ratio of risk factors

	Odds ratio	95% CI	*p* value
Parity	0.44	0.20–0.97	0.04
Age	3.10	1.60–6.04	<0.001

The variables "age" "milk" "alcohol" "caffeine" "meat" "smoking" were generated by summing data for the female subject and her partner because there was a positive correlation between them for these variables.

the subjects in 2000. It is very probable that the blood CB-153 concentrations estimated after so many years would have been subject to gross errors, and this was possibly a major reason why these investigators failed to find any association between blood CB-153 concentrations and TTP.

It may be worth pointing out that the present result indicates a lack of association between TTP and methylmercury exposure in Japanese. In other words, methylmercury exposure at the present level does not affect human fecundity. This may be valuable information in the hazard identification of methylmercury.

In summary, we could not obtain reproducible results showing a positive relationship between fish consumption and TTP in this cohort study using hair mercury levels as an indicator of fish consumption, although such a relationship had been indicated in our earlier study. We will extend our survey by including direct indicators that can reflect exposure level to OCs during the duration of the TTP to characterize the health risk posed by fish consumption, which is important for Japanese and other fish-eating populations.

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Short Communication

No association between green tea and prostate cancer risk in Japanese men: the Ohsaki Cohort Study

N Kikuchi*,[1], K Ohmori[1], T Shimazu[1], N Nakaya[1], S Kuriyama[1], Y Nishino[2], Y Tsubono[3] and I Tsuji[1]

[1]*Division of Epidemiology, Department of Public Health and Forensic Medicine, Tohoku University Graduate School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8575, Japan;* [2]*Department of Epidemiology, Miyagi Cancer Center, Natori 981-1293, Japan;* [3]*Division of Health Policy, Tohoku University School of Public Policy, Sendai 980-8576, Japan*

In a prospective study of 19 561 Japanese men, green-tea intake was not associated with a lower risk of prostate cancer (110 cases), the multivariate hazard ratio for men drinking ⩾5 cups compared with <1 cup per day being 0.85 (95% confidence interval 0.50–1.43, trend $P=0.81$).

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Although laboratory studies have suggested a protective effect of green-tea polyphenols against development of prostate cancer in animal models (Gupta *et al*, 1999; Saleem *et al*, 2003), few epidemiological studies have examined the association. A case–control study in China found that green-tea intake was associated with a lower risk of prostate cancer (Jian *et al*, 2004), whereas a prospective study of Japanese Americans in Hawaii and a case–control study in Japan found no such association (Severson *et al*, 1989; Sonoda *et al*, 2004). The age-standardised incidence of prostate cancer is low in Japan (12.7 per 100 000), being approximately one-tenth of that in the US (Parkin, 2002). Green-tea consumption per capita in Japan is the highest in the world (International Tea Committee, 2004). One reason for the low incidence of prostate cancer in Japan may be the high consumption of green tea. We therefore examined the association between green-tea consumption and prostate cancer incidence among men in the Ohsaki Cohort Study conducted in rural Japan.

MATERIALS AND METHODS

The details of the Ohsaki Cohort Study have been described previously (Tsuji *et al*, 1999; Anzai *et al*, 2005). Briefly, this prospective cohort study was started in 1994 and included 26 481 men aged 40–79 years living in 14 municipalities of Miyagi Prefecture (95% response rate) (Anzai *et al*, 2005). The study used a self-administered questionnaire that included items about the frequency of consumption of beverages (coffee, green tea, black tea) and food items, as well as alcohol drinking, smoking and other health-related lifestyle factors. We asked the subjects about their frequency of green-tea consumption according to five categories: never, occasionally, 1–2 cups per day, 3–4 cups per day and 5 or more cups per day. The validity of green-tea consumption was assessed by calculating Spearman correlation coefficients between the 12-day dietary records and the 40-item food-frequency questionnaire. The age- and energy-adjusted Spearman correlation coefficient in men was 0.71 (Ogawa *et al*, 2003). After exclusion of subjects with missing responses or with a prior history of cancer, 19 561 subjects remained. We followed up the vital and residential status of the subjects using population registries from 1 January 1995 to 31 December 2001. Reference to population-based cancer registries identified 110 incident cases of prostate cancer (7 years of follow-up with 121 543 person-years). During the study period, there was no mass screening programme for prostate cancer in this area.

We combined the lower two categories of green-tea consumption into the single category 'less than one cup per day' because of the small number of subjects in each category. We estimated hazard ratios (HRs) and the 95% confidence interval (CI) of prostate cancer incidence according to green-tea consumption, using the Cox proportional hazards model with adjustment for age and potential confounders. *P*-values for the test of linear trend were calculated by treating the green-tea consumption category as an ordinal variable. All *P*-values were two-tailed. This study had approximately 80% statistical power, with a two-sided α-error level of 5%, in detecting a true HR of 0.75 among the highest *vs* lowest categories of green-tea consumption.

RESULTS

Table 1 shows the characteristics of the subjects according to green-tea consumption. Subjects with a higher green-tea intake tended to be older, to have a higher calorie intake, to consume calcium and fish more frequently, and to drink coffee less frequently.

We found no significant association between green-tea consumption and the risk of prostate cancer. Multivariate HRs for prostate cancer associated with drinking 1–2, 3–4 and 5 or more cups of green-tea per day, as compared with less than one cup per day, were 0.77 (95% CI 0.42–1.40), 1.15 (0.69–1.94), and 0.85 (0.50–1.43), respectively (trend $P=0.81$) (Table 2). Exclusion of

*Correspondence: Dr N Kikuchi; E-mail: nkikuchi@mail.tains.tohoku.ac.jp
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Table 1 Characteristics of the subjects according to green-tea consumption

	Green-tea consumption (cups per day)			
Characteristic	**<1**	**1 or 2**	**3 or 4**	**≥5**
No.	5982	4460	3397	5122
Age (years), means ± s.d.	57.8 ± 10.7	58.0 ± 10.9	60.5 ± 10.4	61.9 ± 9.9
Smoking (%)				
Never	20.5	19.1	19.1	16.9
Past	24.7	24.0	27.5	27.8
Current	54.8	56.9	53.4	55.3
Alcohol drinking (%)				
Never	16.4	14.6	15.2	18.3
Past	10.5	9.8	10.2	11.5
Current	73.1	75.6	74.6	70.2
Body mass index (%)				
<18.5	8.4	7.6	6.4	7.6
18.5–24.9	65.3	67.0	68.6	67.7
≥25.0	26.3	25.4	25.0	24.7
Daily calorie intake (kcal day^{-1}), means ± s.d.	1776 ± 614	1809 ± 602	1847 ± 589	1902 ± 592
Daily calcium intake (mg day^{-1}), means ± s.d.	373 ± 163	400 ± 163	423 ± 160	457 ± 160
Walking duration (%)				
At least 1 h day^{-1}	49.6	48.1	45.8	48.6
Under 1 h day^{-1}	50.4	51.9	54.2	51.4
Meat consumption (%)				
Few	28.6	24.8	26.1	29.0
1–2 times/month	48.8	50.9	50.2	47.5
1–2 or more times/week	22.6	24.3	23.7	23.5
Fish consumption (%)				
Few or 1–2 times/week	35.5	31.7	26.8	22.1
3–4 times/week	32.6	34.8	36.4	33.8
Daily	31.9	33.5	36.8	44.1
Coffee consumption (%)				
Never	23.4	17.3	19.4	23.1
Occasionally	33.0	32.3	38.2	41.3
1–2	29.1	37.1	31.1	23.8
≥3	14.5	13.3	11.3	11.8
Black tea consumption (%)				
Never	68.8	63.4	63.2	66.3
Occasionally	29.1	30.6	32.6	30.2
1–2	1.7	5.3	2.5	2.1
≥3	0.4	0.7	1.7	1.4

$n = 19561$. s.d. denotes standard deviation.

Table 2 HRs and 95% CIs of prostate cancer according to green-tea consumption

	Green-tea consumption (cups per day)				
Variable	**<1**	**1 or 2**	**3 or 4**	**≥5**	**Trend *P***
No. of cases	29	18	31	32	
Person-years	36925	27658	24788	32172	
Age-adjusted HR	1.00	0.79 (0.44–1.43)	1.26 (0.76–2.09)	0.90 (0.55–1.50)	0.96
Multivariate HR[a]	1.00	0.77 (0.42–1.40)	1.15 (0.69–1.94)	0.85 (0.50–1.43)	0.81

HR = hazard ratio; CI = confidence interval. [a]Mulitivariate HR was adjusted for age (in years), body mass index (<18.5, 18.5–24.9 and ≥25.0), alcohol consumption (never, former and current drinking), smoking status (never, former, and current smoking), marital status (marriage at age <25, 25–29, ≥30, unmarried, separated or divorced), daily calorie intake (continuous), daily calcium intake (tertile), walking duration (<1 h day^{-1}, and ≥1 h day^{-1}), consumption frequencies of black tea and coffee (never, occasionally, 1–2, and >3 cups per day), consumption frequencies of meat (few, 1–2 times/month, 1–2 or more times/week) and consumption frequencies of fish (few or 1–2 times/week, 3–4 times/week, daily).

Epidemiology

prostate cancer cases diagnosed in the first 3 years of follow-up did not substantially change the results.

When the data were stratified according to age, smoking, alcohol drinking, body mass index, frequencies of meat and fish consumption, and frequencies of coffee and black tea consumption, there was no association between green-tea consumption and the risk of prostate cancer.

We also examined the relationship between consumption of black tea or coffee and the risk of prostate cancer. The multivariate HRs (95% CI) compared with men who never drank black tea were 1.34 (0.77–2.34) for those drinking black tea occasionally and 0.60 (0.13–2.68) for those drinking one or more cups per day (trend $P = 0.78$). The corresponding HRs for coffee were 0.60 (0.35–1.05) and 0.67 (0.38–1.19) (trend $P = 0.27$). Our results were consistent with the judgment of the World Cancer Research Fund that consumption of black tea or coffee has no relationship with the risk of prostate cancer (World Cancer Research Fund, 1997).

DISCUSSION

This is the first prospective cohort study of green-tea consumption and prostate cancer incidence in Japan. We found no association between green-tea consumption and prostate cancer incidence among Japanese men, who consume green tea much more frequently than men in Western countries. Our results conflicted with those of a case–control study in China (Jian *et al*, 2004), but agreed with those of a prospective study in Hawaii and a case–control study in Japan showing no association between green-tea consumption and prostate cancer incidence (Severson *et al*, 1989; Sonoda *et al*, 2004).

Our study had several methodologic advantages over previous studies of the subject. We recruited subjects from the general population, and there was a large variation in green-tea consumption among our subjects. In addition, we assessed the consumption of green-tea and other variables before cases of prostate cancer were diagnosed, thus avoiding recall bias. The questionnaire used to measure green-tea consumption had a reasonably high level of validity and reproducibility.

As a potential limitation of the study, we could not specifically examine the effect of very high consumption of green tea because the highest category in our questionnaire was five or more cups per day. However, the validation study of our food frequency questionnaire found that 53% of the subjects who reported consuming five or more cups per day actually consumed seven or more cups per day according to 12-day diet records (Ogawa *et al*, 2003). It is therefore unlikely that we failed to detect a decreased risk of prostate cancer among the subjects consuming very large amounts of green tea.

In conclusion, this prospective cohort study conducted in rural Japan showed no association between consumption of green tea, coffee or black tea and the risk of prostate cancer.

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