Demographic, behavioral, dietary, and socioeconomic characteristics related to persistent organic pollutants and mercury levels in pregnant women in Japan.

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Abstract

Persistent organic pollutants and mercury are known environmental chemicals that have been found to be ubiquitous in not only the environment but also in humans, including women of reproductive age. The purpose of this study was to evaluate the association between personal lifestyle characteristics and environmental chemical levels during the perinatal period in the general Japanese population. This study targeted 322 pregnant women enrolled in the Hokkaido Study on Environment and Children's Health. Each participant completed a self-administered questionnaire and a food-frequency questionnaire to obtain relevant information on parental demographic, behavioral, dietary, and socioeconomic characteristics. In total, 58 non-dioxin-like polychlorinated biphenyls, 17 dibenzo-p-dioxins and –dibenzofuran, and 12 dioxin-like polychlorinated biphenyls congeners, perfluorooctane sulfonate, perfluorooctanoic acid, and mercury were measured in maternal samples taken during the perinatal period. Linear regression models were constructed against potential related factors for each chemical concentration. Most concentrations of environmental chemicals were correlated with the presence of other environmental chemicals, especially in the case of non-dioxin-like polychlorinated biphenyls and, polychlorinated dibenzo-p-dioxins and -dibezofurans and dioxin-like polychlorinated biphenyls which had similar exposure sources and persistence in the body. Maternal smoking and alcohol habits, fish and beef intake and household income were significantly associated with concentrations of environmental chemicals. These results suggest that different lifestyle patterns relate to varying exposure to environmental chemicals.

Keywords: persistent organic pollutants, mercury, pregnant women, biomarkers, POPs

1. Introduction

Polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and -dibezofurans (PCDDs/PCDFs), perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA)—categorized as persistent organic pollutants—and mercury (Hg) are known environmental chemicals that have been detected ubiquitously in animal samples and the environment. Exposure to environmental chemicals during prenatal and neonatal periods, which are considered windows of vulnerability for fetuses, may cause various toxicities including carcinogenicity, teratogenicity, endocrine, immune, and reproductive disruption, and neurobehavioral effects (Clarkson and Magos, 2006; Olsen et al., 2009; Todaka et al., 2010; Wigle et al., 2008).

Epidemiological studies of Asian, European, and US populations have revealed that environmental chemical levels measured in maternal samples were associated with demographic, behavioral, dietary, and socioeconomic characteristics. Fish and seafood are the main dietary sources of PCB and PCDDs/PCDFs exposure in Japan, Taiwan, Nordic countries, and Italy (Arisawa et al., 2011); whereas, meat products, dairy products and fish are the main dietary sources in the US, The Netherlands, and Germany (Larsen, 2006). Potential exposure sources of PFOS and PFOA were reported to be fish and marine mammals, red meat, animal fat, tap (drinking) water, and household dust in Spain, Norway, and Denmark (D'Hollander et al., 2010; Haug et al., 2011). Many reports to date have also found fish/seafood consumption responsible for bio-accumulated methylmercury in humans (Clarkson and Magos, 2006; Kim et al., 2008; Ramon et al., 2008). Consequently, it is plausible that the presence of exposure sources and their contribution to whole body burden levels of environmental chemicals would vary according to the specific characteristics of populations in different countries or regions (Glynn et al., 2007; Halldorsson et al., 2008; Kim et al., 2008; Ramon et al., 2008; Sonneborn et al., 2008; Brauner et al., 2011; Ibarluzea et al., 2011).

The elimination rate of toxic substances as a reflection of internal metabolism is an effective way to detect body burden levels of environmental chemicals..Tobacco smoking and alcohol habits are considered behavioral factors related to altered elimination rates of environmental chemicals. For example, tobacco smoking induces increased expression of dioxin-metabolizing enzymes, such as cytochrome P450 (CYP) 1A2, leading to enhanced elimination of PCDDs/PCDFs and dioxin-like PCBs (DL-PCBs) (Milbrath et al., 2009). Animal and human studies have also demonstrated that fluorinated organic compounds can regulate CYP enzymes (Ishibashi et al., 2008; Narimatsu et al., 2011).

To date, limited epidemiological studies have been conducted in Japan among

pregnant women with no history of accidental poisoning in Japan. Some studies found that PCBs and PCDDs/PCDFs in maternal samples increased with maternal age, alanine aminotransferase levels and alcohol intake, as well as decreased with maternal history of delivery and smoking (Arisawa et al., 2011; Tajimi et al., 2005; Nakamura et al., 2008). However, no study has assessed maternal smoking and alcohol habits during the pre-pregnancy periods, which is considered an important period because chemicals that have a long half-life could be influenced by lifestyle factors during the entire perinatal period. There have also been no current studies to evaluate associations between background exposure levels of environmental chemicals even though certain chemical levels could be correlated with the presence of other chemicals in the human body. This information could help in estimating the magnitude of body burden levels after exposure to various chemicals.

Thus, the purpose of this study was to evaluate associations between concentrations of individual chemicals including non-dioxin-like PCBs (NDL-PCBs), PCDDs/PCDFs and DL-PCBs, PFOS, PFOA and Hg and the potential factors responsible for their varied elimination rates and exposure sources in the general Japanese population.

2. Materials and Methods

2.1 Study population

We enrolled 514 Japanese women at 23–35 weeks gestation who were visiting the Sapporo Toho Hospital to take part in the Hokkaido Study on Environment and Children's Health Study (Kishi et al., 2011) between July 2002 and September 2005 (Supplementary Figure 1). In their last trimester, the subjects filled out a self-administered questionnaire regarding the following parental information: tobacco smoking and alcohol habits during pre- and post-pregnancy; frequency of food consumption during pregnancy of items such as shoreline fish (e.g., saury, Pacific herring, mackerel), pelagic fish (e.g., tuna, bonito, salmon), beef, pork, chicken, milk, and eggs; education level; and household income. Estimated intake value for alcohol (g/day) was calculated from a modified self-administered questionnaire about frequency and type of alcohol consumption (Washino et al., 2009).

From enrollment to delivery, 10 subjects dropped out because of intrauterine growth retardation (2), hospital transfer (1), or voluntary withdrawal (7). The medical records for the remaining 504 mother–newborn pairs were used to obtain data on maternal height and weight before pregnancy. To obtain information on maternal fish

intake throughout pregnancy, we contacted subjects within 5 days of delivery. They completed part of a food-frequency questionnaire (FFQ) and provided information about intake frequency and portion size for 28 fish and seafood items and their estimated total fish intake (g/day) was calculated as previously described (Yasutake et al., 2003) (Supplementary Table 1). We were not able to contact 74 subjects because of poor health conditions immediately after delivery. Subjects also provided a sample of their hair for Hg measurements and information on their past history of having their hair permed. This study was conducted with written informed consent from all subjects and was approved by the institutional ethics board for epidemiological studies at the Hokkaido University Graduate School of Medicine.

2.2 Experimental and exposure assessment

A 40-mL blood sample was taken from the maternal peripheral vein in the last trimester, except in those subjects with pregnancy-related anemia, from whom blood samples were taken immediately after delivery. All blood samples were stored at −80°C. NDL-PCBs (Supplementary Table 2) and, PCDDs/PCDFs and DL-PCBs levels (Supplementary Table 3) in maternal blood were detected by high-resolution gas chromatography/high-resolution mass spectrometry equipped with a solvent-cut large-volume injection system at the Fukuoka Institute of Health and Environmental Sciences as previously described (Iida and Todaka, 2003; Todaka et al., 2003; Todaka et al., 2008). NDL-PCBs and, PCDDs/PCDFs and DL-PCBs levels were adjusted by total lipid content (pg/g lipid)(Todaka et al., 2003). Toxic equivalent (TEQ) values were calculated by multiplying the concentration of each individual congener of PCDDs/PCDFs and DL-PCBs by its specific toxic equivalency factor value (Van den Berg et al., 2006). PFOS and PFOA levels in maternal serum were detected by column-switching liquid chromatography–tandem mass spectrometry at Hoshi University as previously described (Inoue et al., 2004a; Inoue et al., 2004b; Nakata et al., 2005a; Nakata et al., 2005b). Values below the detection limit were assigned as 50% of the detection limit. The remaining samples were not analyzed owing to unavailable or insufficient sample volumes for measurement. Total Hg levels were detected in the 1-cm hair fiber closest to the scalp (0.7–1.2 mg) by the oxygen combustion-gold amalgamation method using an MD-1 atomic absorption detector (Nippon Institute, Co., Ltd., Osaka) at the National Institute for Minamata Disease as previously described (Yasutake et al., 2003). Total hair Hg concentration is a convenient biomarker for methylmercury exposure because >90% of total hair Hg is methylmercury, which covalently binds to cysteines in hair proteins (Clarkson and Magos, 2006). Finally, 58

NDL-PCBs, 12 DL-PCBs and 17 PCDDs/PCDFs congeners—were detected in 426 blood samples. PFOS and PFOA, and total Hg were detected in 447 sera samples and 430 hair samples, respectively.

2.3 Statistical analysis

In total, 322 subjects that had complete data about concentration of environmental chemicals and personal characteristics were included in the statistical analyses. Subjects were divided into four categories for each of maternal age, BMI, blood sampling period, and fish intake during pregnancy as shown in Table 1. Spearman's rank test was used to determine correlations between concentrations of environmental chemicals. The Mann–Whitney U-test and Kruskal–Wallis test were used to evaluate simple associations between subject characteristics and the concentrations of each environmental chemical. Linear regression analyses were performed to evaluate associations between concentrations of environmental chemicals and subject characteristics. Because of skewed distributions in these concentrations, $\log_{10}$-transformed values were used for linear regression analysis. Linear regression models were constructed for explanatory variables that had previously been reported as related to concentrations of environmental chemicals or that were significantly associated with these concentrations by bivariate analysis in this study. Backward stepwise regression was used to eliminate those variables with a p-value >0.1.

Subgroup analyses were performed to confirm significant associations between maternal smoking history and alcohol consumption during pregnancy and concentrations of each environmental chemical. Duration of maternal smoking (years) was used as a continuous explanatory variable in subgroup analyses of subjects with a history of smoking. Alcohol intake levels (g/day), after categorization into four groups according to their quartile distribution, were used in subgroup analyses of a subjects' alcohol consumption during pregnancy. Presence of NDL-PCBs and, PCDDs/PCDFs and DL-PCBs congeners were examined in subgroup analyses among alcohol drinkers during pregnancy. Statistical significance was defined as $p < 0.05$. Statistical analyses were performed using SPSS for Windows version 19.0J (SPSS, Inc., USA).

3. Results

Parental characteristics based on the self-administered questionnaire and the FFQ are shown in Table 1. Approximately half (52.2%) of the mothers had a history of tobacco smoking; 17.1% of mothers smoked during the pregnancy. There was a history of alcohol consumption in 73.6% of the mothers, and 30.1% of the mothers consumed

alcohol during pregnancy. Median concentrations of NDL-PCBs, PCDDs/PCDFs and DL-PCBs, PFOS, PFOA, and hair Hg were 95.1 ng/mL lipid, 13.8 TEQ pg/g lipid, 5.00 ng/mL, 1.30 ng/mL, and 1.39 μg/g, respectively (Table 2). Table 3 shows the correlations between concentrations of individual environmental chemicals. The strongest correlation was found between NDL-PCBs and, PCDDs/PCDFs and DL-PCBs ($r = 0.80$, $p < 0.01$). In univariate analyses of maternal levels of environmental chemicals in relation to maternal characteristics, levels of environmental chemicals were significantly associated with maternal age at delivery, parity, blood sampling period, education level, smoking and alcohol habits, fish intake, frequency of food consumption, and annual household income ($p < 0.05$; Table 4).

The linear regression models in Figure 1 show the potential relationship between various factors and maternal blood concentrations of NDL-PCBs and, PCDDs/PCDFs and DL-PCBs, PFOS, and PFOA, and total hair Hg. Significant positive associations with each $\log_{10}$-transformed concentration of environmental chemicals were observed for maternal age, maternal alcohol consumption during pregnancy, fish intake, pelagic fish intake, beef intake, and household income (Supplementary Table 4). Significant negative associations with each $\log_{10}$-transformed concentration of environmental chemicals were observed for multiparous subjects, smoking history, and blood sampling period (Supplementary Table 4). In the subgroup analyses of the 168 subjects with a history of smoking, the duration of tobacco smoking was inversely associated with $\log_{10}$-transformed PFOS values [Figure 2; −0.08 (−0.15, −0.02)]. With additional adjustment for maternal age, statistical significance remained. In the subgroup analysis of the 97 subjects who reported drinking alcohol during their pregnancy, there was no significant association between NDL-PCBs and, PCDDs/PCDFs and DL-PCBs and their congeners with alcohol intake for any quartile as well as across quartiles (Table 5; Supplementary Table 5).

4. Discussion

4.1 Correlations between concentrations of environmental chemicals

NDL-PCBs and, PCDDs/PCDFs and DL-PCBs are reported to have high lipophilicities and the biological half-life of most their congeners ranges from a few years to approximately 20 years (Todaka et al., 2010). Perfluoroalkyl acids (PFAAs) are reported to distribute mainly in blood serum and the liver as a result of protein fraction binding (Karrman et al., 2010), and the half-lives of PFOS and PFOA are estimated to be 3.8 and 5.4 years, respectively (Olsen et al., 2009). Methylmercury binds to

hemoglobin in the blood, and its half-life is estimated to be 2 months (Clarkson and Magos, 2006). The degrees of correlations between environmental chemicals could correspond to differences in their exposure sources as well as their individual pharmacokinetics. Almost all of the environmental chemicals, with the exception of relationships between PFOS and PCBs, PFOA and PCBs, and Hg and PFOA, were significantly correlated, implying that concentrations of certain environmental chemicals could be used to estimate the magnitude of exposure among the general population in Japan to other environmental chemicals, especially those from similar exposure sources and with similar persistence in the body.

4.2 Tobacco smoking

Maternal smoking history was significantly related to a decline in concentrations of PCDDs/PCDFs and DL-PCBs, and PFOS in this study. A previous study reported that tobacco smoking lead to a decrease in PCDDs/PCDFs and DL-PCBs levels because of increased expression of dioxin-metabolizing enzymes after activation of the aryl hydrocarbon receptor (Milbrath et al., 2009). PFAAs are known to activate peroxisome proliferator-activator receptor (PPAR), and a study in wild animals suggested the possibility that a signaling pathway exists between receptor PPAα and CYP that promotes elimination of PFAAs from the body after PFAA exposure (Ishibashi et al., 2008). Previous epidemiological studies reported inconsistent relationships between PFAA levels and smoking status among pregnant women when categorized by history or current smoking status (Halldorsson et al., 2008; Halldorsson et al., 2012; Jain, 2013; Ode et al., 2013). In a Swedish study, maternal cotinine levels among current smokers were not associated with PFOA and PFOS plasma levels; in fact, PFOA and PFOS plasma levels were significantly lower than those of subjects who had never smoked (Ode et al., 2013). Ode discussed that these results could reflect differences in lifestyle patterns between smokers and non-smokers that were associated with sources of PFOA and PFOS exposure or an enhanced elimination rate of these environmental chemicals in smokers. Our study is the first study to indicate an inverse association between PFOS concentrations and the duration of tobacco smoking using a linear regression model adjusted for confounding factors. This result supports previous studies and suggests that a smoking habit may lead to enhanced elimination rate of not only PCDDs/PCDFs and DL-PCBs but also PFOS through activation of PPAα and CYP.

4.3 Alcohol consumption

Our results showed that mothers who drank alcohol during pregnancy had

higher blood concentrations of NDL-PCBs and, PCDDs/PCDFs and DL-PCBs. In a previous Japanese study that included women of reproductive age, two possible explanations for this positive association between PCDDs/PCDFs and DL-PCBs and alcohol consumption were proposed. The first is that alcohol intake likely affects hepatic drug-metabolizing enzymes, which could result in slowed elimination of these environmental chemicals. The second is that alcohol intake may indicate a greater likelihood of rich, fatty food consumption, which may result in increased PCDDs/PCDFs and DL-PCBs levels (Arisawa et al., 2011). In this study, no significant associations were found across alcohol-intake quartiles and concentrations of NDL-PCBs and, PCDDs/PCDFs and DL-PCBs, and congeners among alcohol drinkers during pregnancy. However, concentrations NDL-PCBs and, PCDDs/PCDFs and DL-PCBs in maternal drinkers were higher than those of women who did not drink during pregnancy. Because of their long half-lives, NDL-PCBs and, PCDDs/PCDFs and DL-PCBs could be influenced by drinking in the pre-pregnancy period as well as that of pregnancy, on the assumption that maternal alcohol intake could affect the elimination rate of these chemicals. However, in this study, history of alcohol consumption had no association with NDL-PCBs and, PCDDs/PCDFs and DL-PCBs levels. Therefore, maternal alcohol consumption may reflect subsequent lifestyle patterns during pregnancy that increase concentrations of NDL-PCBs and, PCDDs/PCDFs and DL-PCBs, rather than indicating an effect on hepatic drug-metabolizing enzymes.

4.4 Food intake

We found that meat, especially beef intake, may be an important exposure source of NDL-PCBs in Japan, similar to that in the US and the Europe (Larsen, 2006). In a Japanese food market study, meat provided the second highest contribution to total daily dietary intake of PCDDs/PCDFs and DL-PCBs (Sasamoto et al., 2006). Our results indicated that fish/seafood, especially pelagic fish, may be an important exposure source for Hg. This is supported by another study that showed that large predatory fish were the largest contributor to total hair Hg among pregnant women in Japan (Yaginuma-Sakurai et al., 2009).

4.5 Other related factors

In agreement with previous studies, a history of parity was associated with decreasing concentrations of NDL-PCBs and, PCDDs/PCDFs and DL-PCBs, PFOS and PFOA, suggesting that reproductive events could play a role in elimination of environmental chemicals from the maternal body (Milbrath et al., 2009; Olsen et al.,

2009). PFOS and PFOA were inversely associated with gestational age at the time of blood sampling, possibly due to the dilutional effect of plasma volume expansion, especially after the last trimester (Glynn et al., 2007). NDL-PCBs and, PCDDs/PCDFs and DL-PCBs increased with maternal age, which could be explained by previous reports that maternal age might be a good marker for the estimated duration of exposure to chemicals with long half-lives (Milbrath et al., 2009). Hg in hair increased with household income in our study, which is supported by a previous report indicating that high socioeconomic status is related to increased fish consumption, dental amalgams and vaccines, which are all associated with increased exposure to Hg (Tyrrell et al., 2013).

4.6 Strengths and limitations

This study provides useful information on associations between demographic, behavioral, dietary, and socioeconomic characteristics and background concentrations of individual chemicals including NDL-PCBs and, PCDDs/PCDFs and DL-PCBs, PFOS, PFOA and Hg during the perinatal period by liner regression models. These characteristics may also influence the level of fetal exposure to environmental chemicals through effects on maternal exposure levels. However, we did not collect data during maternal breast-feeding despite indications that this is an important determinant in the body burden of environmental chemicals (Milbrath et al., 2009). Further studies are also needed to evaluate etiological mechanisms of maternal smoking on the elimination rate of PFOS mediated by PPAα and CYP activation.

In conclusion, most concentrations of individual NDL-PCBs, PCDDs/PCDFs and DL-PCBs, PFOS, PFOA and Hg were correlated, especially the association between NDL-PCBs and, PCDDs/PCDFs and DL-PCBs, which had similar exposure sources and persistence in the body. PCDDs/PCDFs and DL-PCBs and PFOS decreased with maternal smoking history. NDL-PCBs and, PCDDs/PCDFs and DL-PCBs increased with maternal alcohol consumption during pregnancy. Total hair Hg increased with household income. Beef and fish/seafood intake may be important exposure sources of NDL-PCBs. These results may reflect various lifestyle patterns associated with exposure sources and elimination rates of these environmental chemicals.

Conflicts of Interest

The authors declare they have no competing financial interests. This study was supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of

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Table 1. Subject characteristics (n = 322).

		Mean ± SD	n (%)
Maternal characteristics			
Age at delivery (years)		30.63 ± 4.70	
BMI before pregnancy (kg/m^2)		21.12 ± 3.21	
Parity	≥1		170 (52.8)
Blood sampling period	<28 weeks		19 (5.9)
	28 to <36 weeks		144 (44.7)
	≥36 weeks		70 (21.2)
	After delivery		99 (30.7)
Education level (years)	>12		191 (59.3)
Tobacco smoking history	Yes		168 (52.2)
Tobacco smoking during pregnancy	Smoker		55 (17.1)
Alcohol consumption history	Yes		237 (73.6)
Alcohol consumption during pregnancy	Drinker		97 (30.1)
Alcohol intake (g/day) during pregnancy		0.00 (0.00, 0.46)[a]	
Fish intake (g/day) during pregnancy		50.00 (30.00, 50.00)[a]	
Frequency of food consumption during pregnancy			
Shoreline fish	≥once/week		155 (48.1)
Pelagic fish	≥once/week		178 (55.3)
Beef	≥once/week		86 (26.7)
Eggs	≥once/week		322 (100)
Milk	≥once/week		285 (88.5)
Paternal characteristics			
Tobacco smoking history	Yes		279 (86.7)
Tobacco smoking during their partner's pregnancy	Smoker		225 (69.9)
Annual household income (million yen)	≥5		110 (34.2)

BMI: body mass index

[a]Median (minimum, maximum)

Table 2. Concentrations of environmental chemicals in maternal samples (n = 322)

			Percentile			
	Geometric mean	Minimum	25th	50th	75th	Maximum
NL-PCBs (ng/g lipid)	94.0	16.0	66.0	95.1	130	445
DL-PCBs and PCDDs/PCDFs (TEQ pg/g lipid)[a]	13.5	3.17	9.86	13.8	18.3	42.9
PFOS (ng/mL)	4.78	1.30	3.20	5.00	6.98	14.7
PFOA (ng/mL)	1.20	0.25	0.80	1.30	1.80	5.30
Hair Hg (μg/g)[b]	1.35	0.24	0.96	1.39	1.89	7.55

[a]TEQs were calculated from the individual congener toxic equivalency factor values (Van den Berg et al., 2006).

[b]>90% methylmercury

Table 3. Correlation coefficients between individual environmental chemicals (n = 322)

	PCDDs/PCDFs and DL-PCBs	PFOS	PFOA	Hair Hg
NDL-PCBs	0.80**	0.07	0.10	0.38**
PCDDs/PCDFs and DL-PCBs		0.24**	0.14*	0.30**
PFOS			0.25**	0.12*
PFOA				0.03

*p <0.05, **p <0.01 by Spearman's rank correlation

1 Table 4. Maternal environmental chemical levels in relation to characteristics (n = 322)

Characteristics		NDL-PCBs (ng/g lipid)	PCDDs/PCDFs and DL-PCBs (TEQ pg/g lipd)	PFOS (ng/mL)	PFOA (ng/mL)	Hair Hg (µg/g)
Age at delivery (years)	<25	60.78**	9.80**	5.0	1.4	1.4
	25 to <30	86.0	13.7	5.3	1.2	1.4
	30 to <35	101.3	14.4	5.0	1.4	1.3
	≥35	136.4	16.9	4.2	1.2	1.7
BMI at delivery (kg/m^2)	<18.5	89.7	13.7	5.4	1.4	1.3
	18.5 to <25	97.2	13.9	5.0	1.3	1.4
	25 to <30	108.4	13.4	4.4	1.4	1.2
	≥30	77.3	12.8	4.3	1.2	1.1
Parity	0	101.0	14.6**	5.50**	1.50**	1.4
	≥1	90.8	13.3	4.6	1.0	1.4
Timing of blood sampling	<28 weeks	114.6	16.7	6.4**	1.8**	
	28 to <36 weeks	108.6	13.9	5.6	1.5	
	≥36 weeks	102.1	13.8	4.6	1.2	
	After delivery	108.2	13.5	3.8	1.2	
Education level (years)	≤12	89.4	12.8	4.8	1.3	1.4
	>12	99.2	14.1	5.3	1.4	1.4
Tobacco smoking history	No	101.0	15.2**	5.30**	1.4	1.5
	Yes	87.8	12.6	4.7	1.2	1.3
Tobacco smoking during pregnancy	Nonsmoker	96.9	14.1	5.0	1.3	1.4
	Smoker	84.8	12.2	4.8	1.2	1.4
Alcohol consumption history	No	86.0	13.3	4.8	1.2	1.3
	Yes	101.0	14.0	5.0	1.4	1.4
Alcohol consumption during pregnancy	Non-drinker	92.1	13.8	5.0	1.3	1.4
	Drinker	101.0	13.8	5.1	1.3	1.4
Alcohol intake (g/day) during pregnancy	Quartile 1 (<0.73)	0.1	0.0	0.0	0.0	0.1
	Quartile 2 (0.73 to <1.52)	96.0	12.3	4.1	1.2	1.3
	Quartile 3 (1.52 to <3.52)	117.3	13.7	5.6	1.2	1.6
	Quartile 4 (≥3.52)	100.4	15.7	6.2	1.4	1.4
Fish intake (g/day) during pregnancy	Quartile 1 (<25)	101.5*	14.0	4.6	1.3	1.5**
	Quartile 2 (25 to <38.75)	84.8	13.0	5.3	1.4	1.3
	Quartile 3 (38.75 to <50)	101.3	13.8	4.9	1.4	1.4
	Quartile 4 (≥50)	104.1	14.3	5.0	1.2	1.7
Frequency of food consumption during pregnancy						
Shoreline fish	<once/week	87.2	13.3	4.7	1.2	1.28*
	≥once/week	101.0	14.5	5.3	1.3	1.5

Pelagic fish	<once/week	94.8	13.4	4.7	1.3	1.25**
	≥once/week	95.6	14.2	5.4	1.3	1.5
Beef	<once/week	95.0	13.7	5.0	1.3	1.34*
	≥once/week	100.0	14.1	5.2	1.3	1.5
Egg	<once/week	92.6	13.6	4.10**	1.3	1.28**
	≥once/week	95.1	13.8	5.0	1.3	1.4
Milk	<once/week	66.2	11.0*	4.3	1.2	1.3
	≥once/week	99.2	14.1	5.0	1.3	1.4
Paternal characteristics						
Tobacco smoking history	No	97.5	15.1	5.4	1.3	1.3
	Yes	94.7	13.7	5.0	1.3	1.4
Tobacco smoking during their partner's pregnancy	Non-smoker	102.0	15.1*	5.4	1.4	1.4
	Smoker	92.5	13.1	4.8	1.3	1.4
Annual household income (million yen)	<5	91.8**	13.3**	4.7	1.2	1.27*
	≥5	113.0	15.8	5.5	1.5	1.5

1 Values shown are medians. BMI: body-mass index

2 *p <0.05, **p <0.01 by the Mann–Whitney U-test and Kruskal–Wallis test

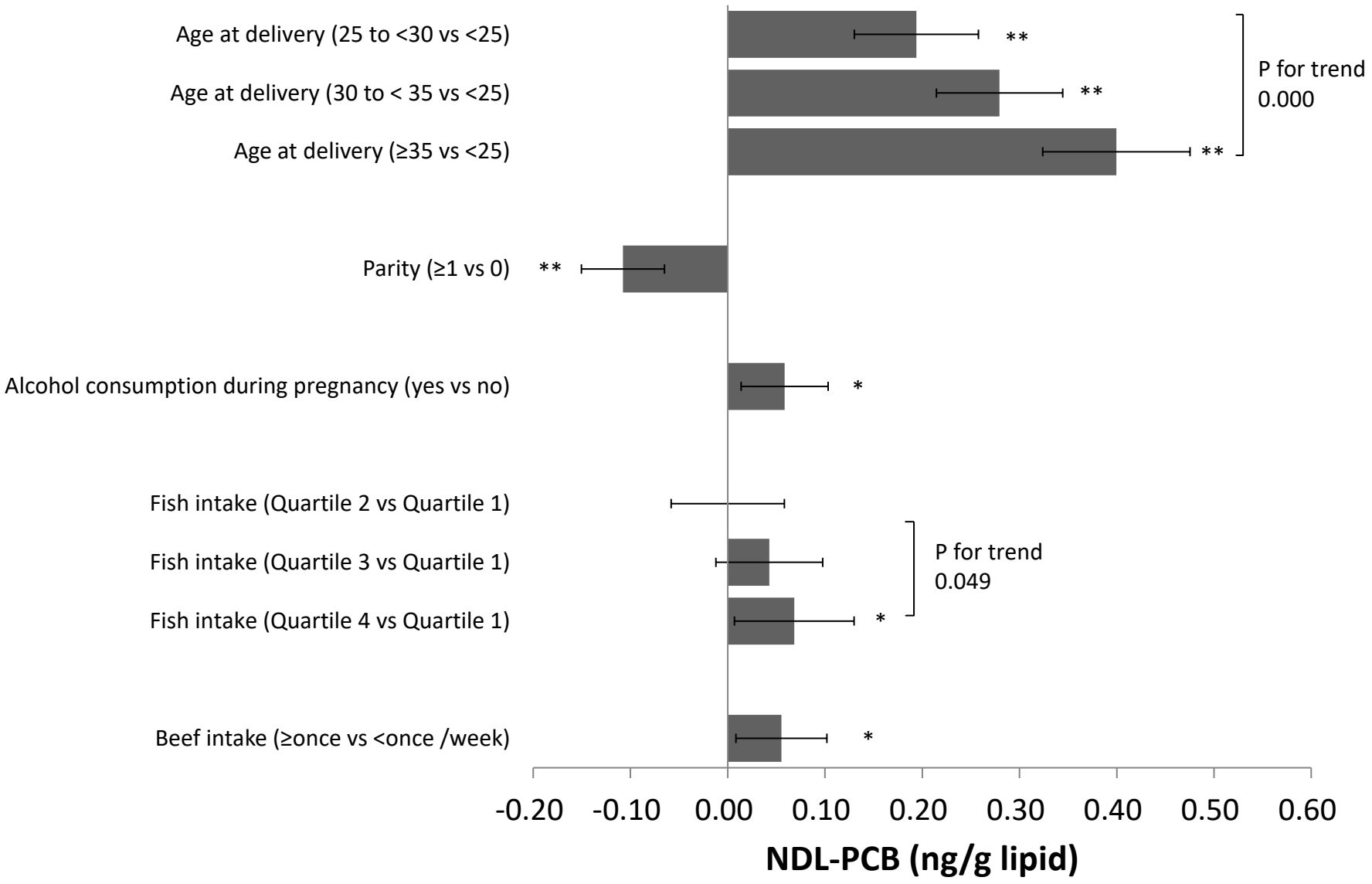
3

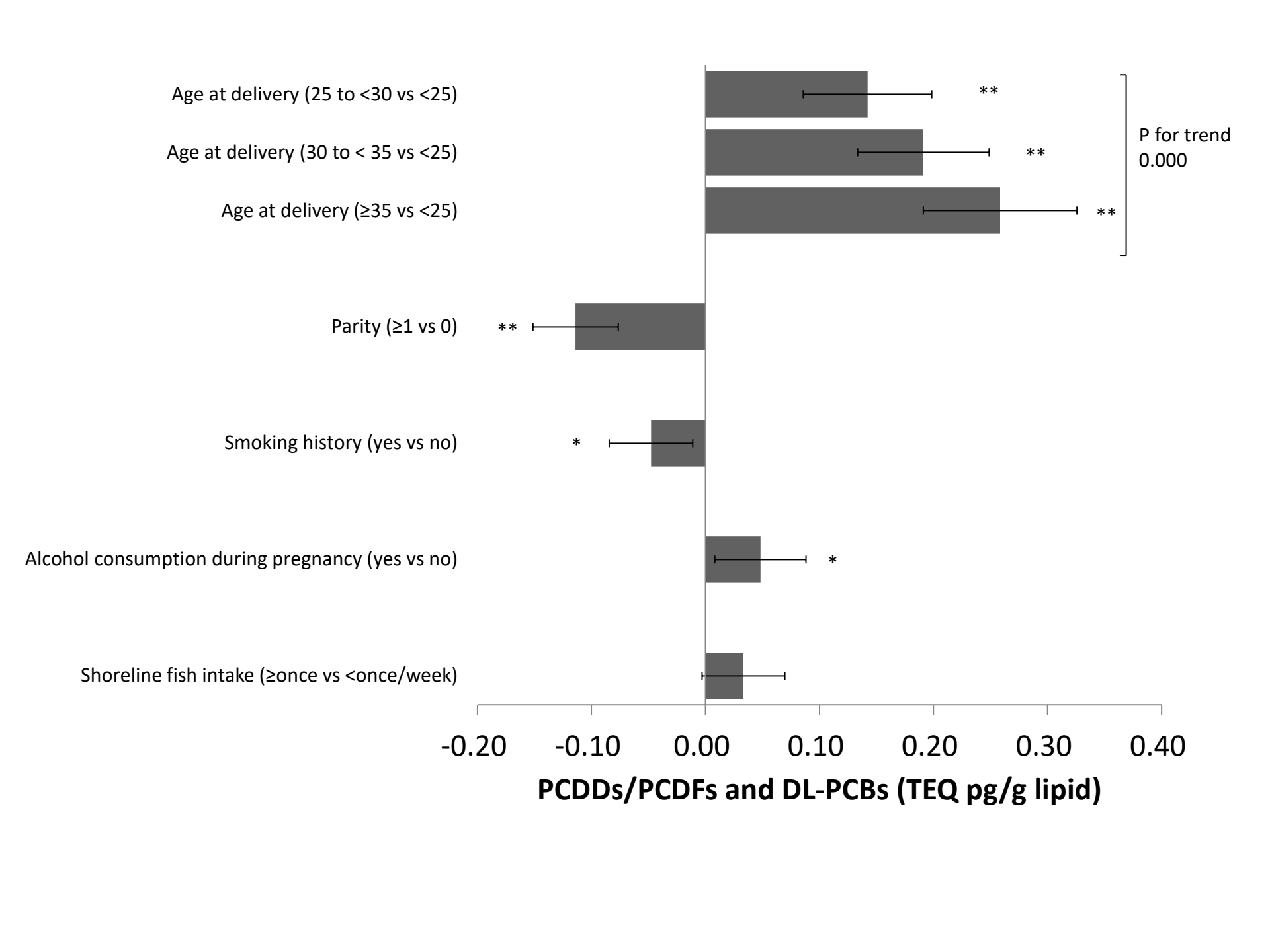
Table 5. Partial regression coefficients (95%CI) for environmental chemical concentrations from mothers who consumed alcohol during pregnancy (n = 97)

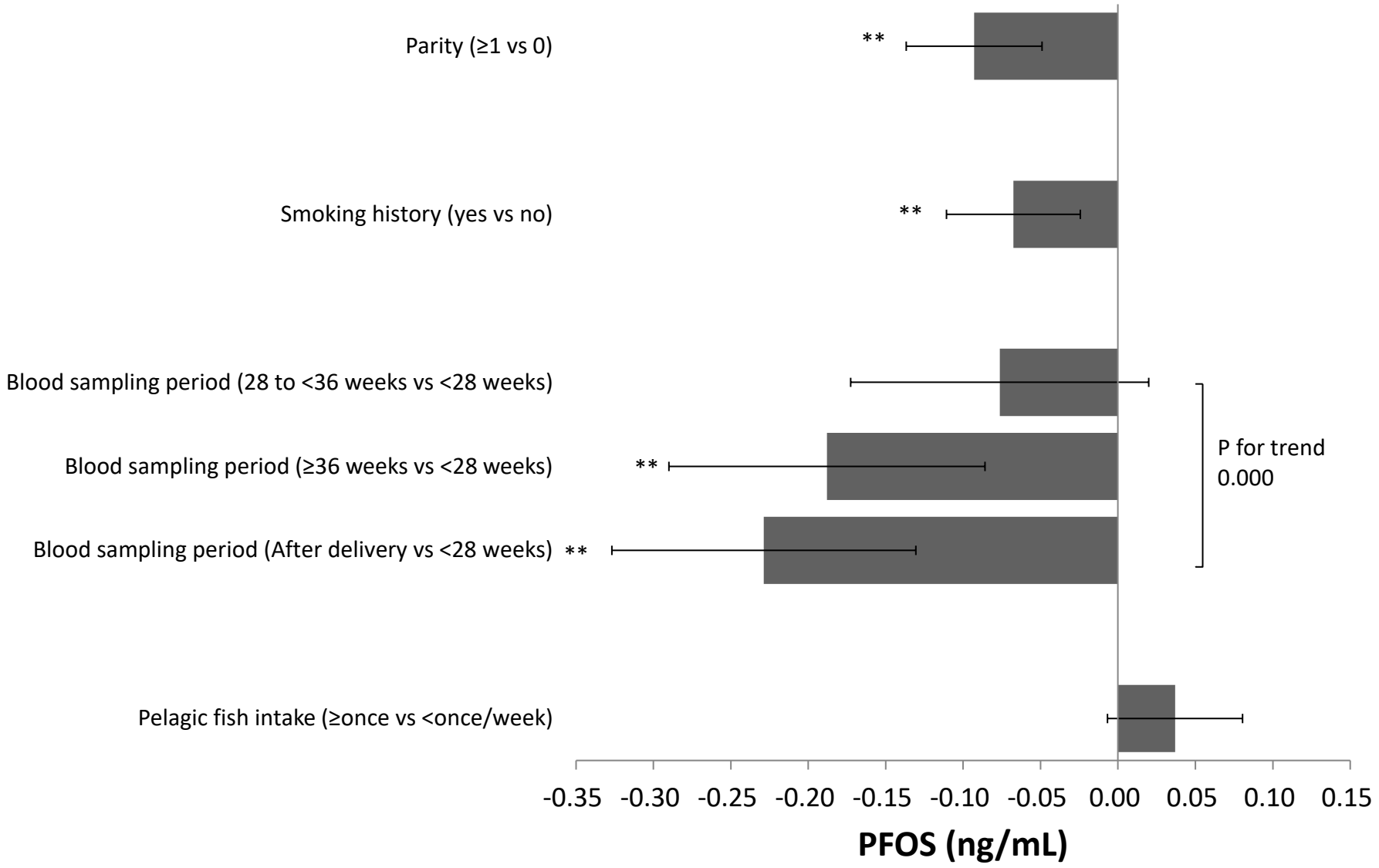
Quartiles by alcohol intake (n, range in g/day)	NDL-PCBs		PCDDs/PCDFs and DL-PCBs	
	B (95%CI)	p for trend[a]	B (95% CI)	p for trend[a]
Quartile 1 (n = 26, <0.73)	reference	0.802	reference	0.404
Quartile 2 (n = 27, 0.73–1.52)	0.07 (−0.05, 0.18)		0.06 (−0.03, 0.16)	
Quartile 3 (n = 24, 1.52–3.52)	0.07 (−0.05, 0.18)		0.08 (−0.02, 0.17)	
Quartile 4 (n = 20, ≥3.52)	0.00 (−0.12, 0.13)		0.04 (−0.07, 0.14)	

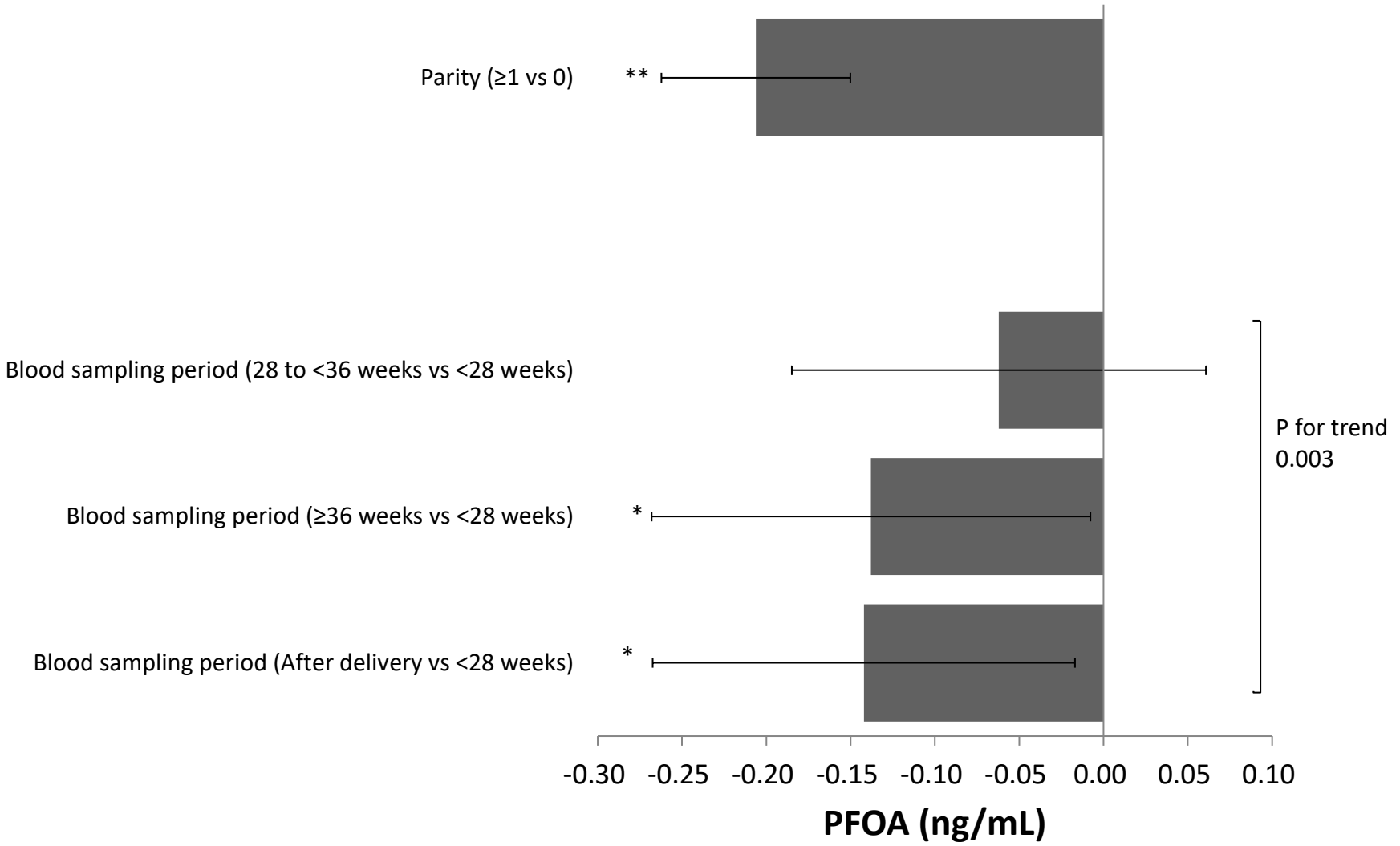
B: partial regression coefficient provides the expected change in the $\log_{10}$-transformed environmental chemical concentrations between quartiles in the regression linear model, adjusted for maternal age, parity, smoking history, fish intake, shoreline fish intake, and beef intake.

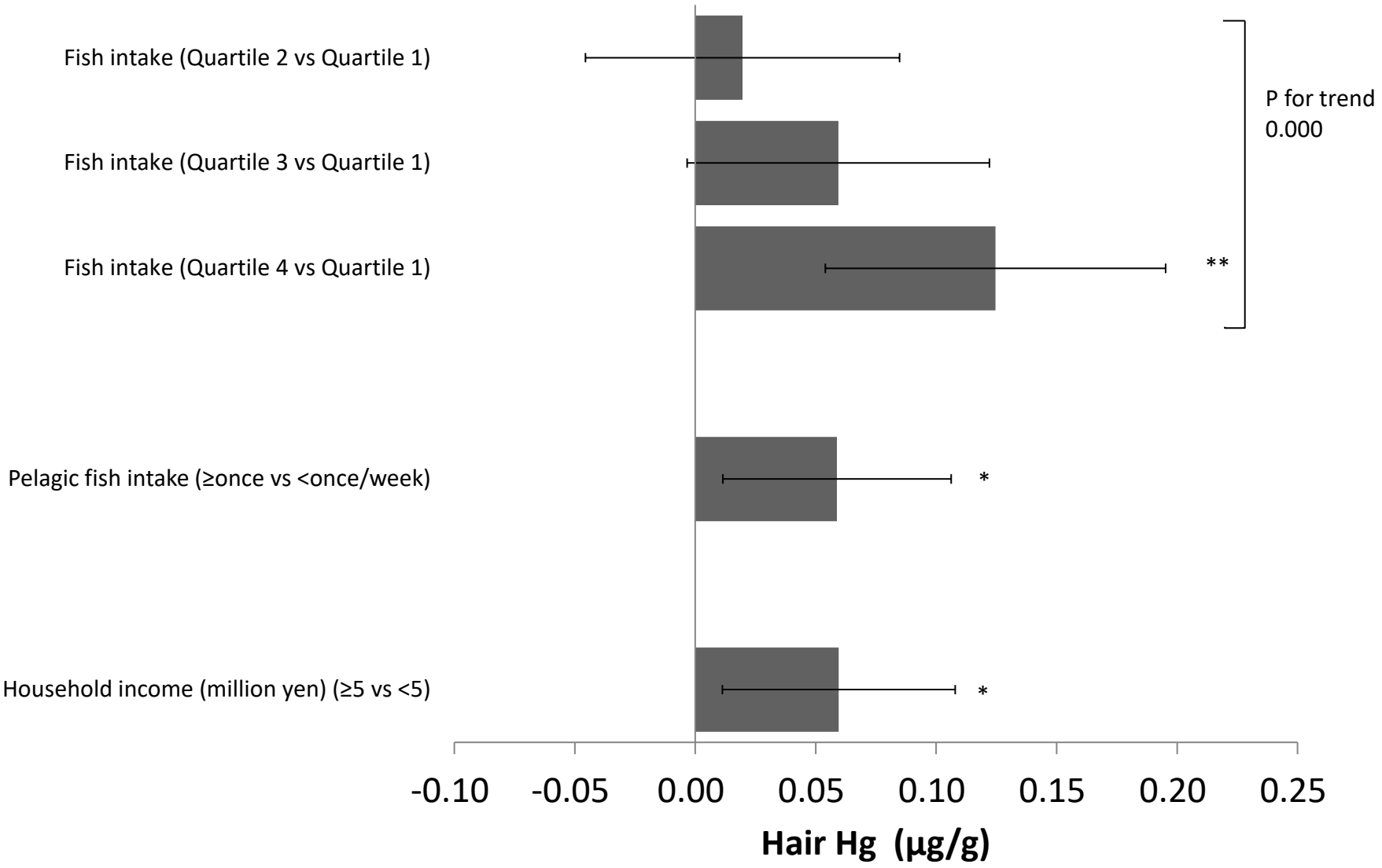
[a]Quartiles are represented as ordinal variables.

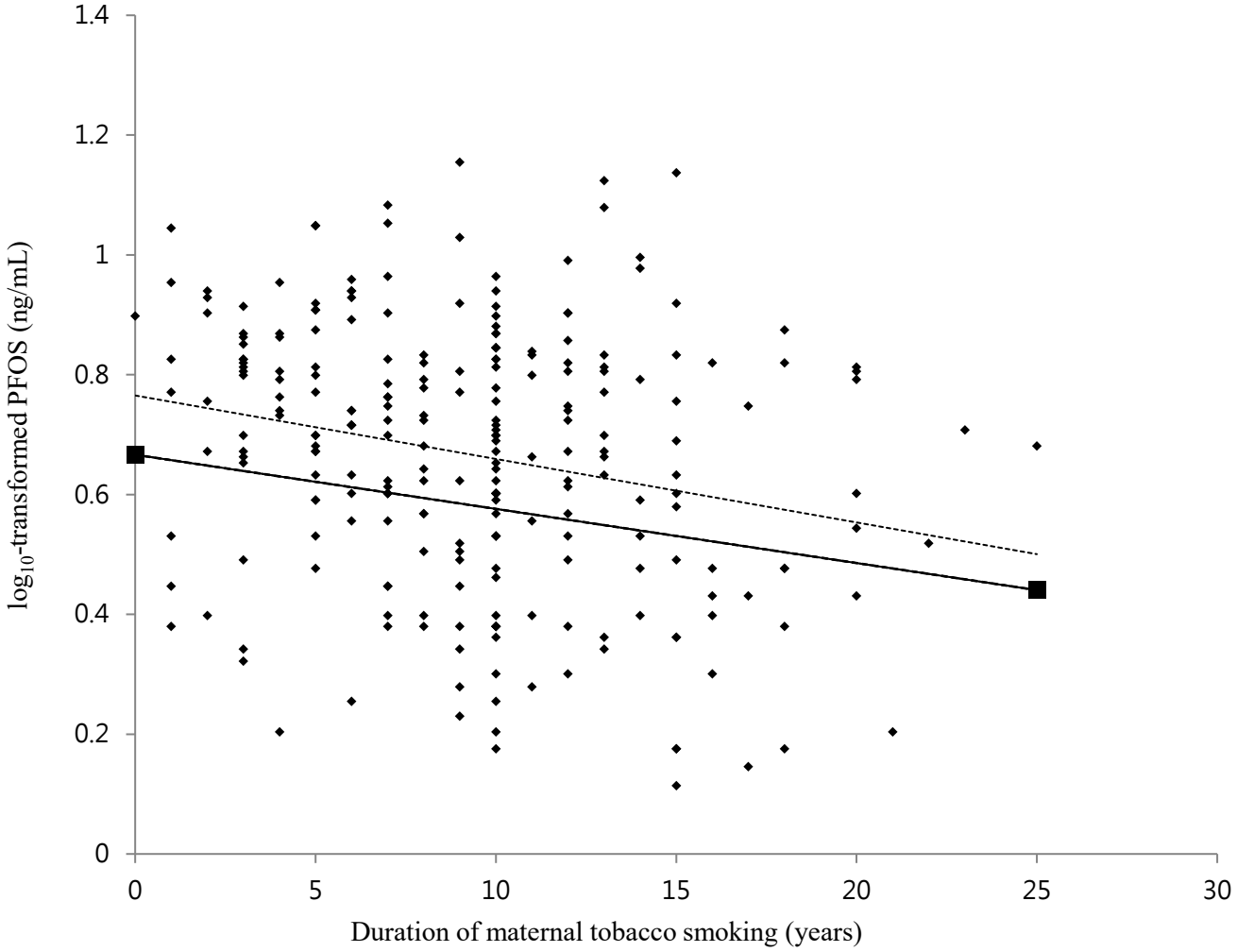

Age at delivery (25 to <30 vs <25)
Age at delivery (30 to < 35 vs <25)
Age at delivery (≥35 vs <25)
**
**
**
P for trend
0.000
Parity (≥1 vs 0)
**
Alcohol consumption during pregnancy (yes vs no)
*
Fish intake (Quartile 2 vs Quartile 1)
Fish intake (Quartile 3 vs Quartile 1)
Fish intake (Quartile 4 vs Quartile 1)
P for trend
0.049
*
Beef intake (≥once vs <once /week)
*
-0.20
-0.10
0.00
0.10
0.20
0.30
0.40
0.50
0.60
NDL-PCB (ng/g lipid)


Age at delivery (25 to <30 vs <25)
Age at delivery (30 to < 35 vs <25)
Age at delivery (≥35 vs <25)
P for trend
0.000
Parity (≥1 vs 0)
Smoking history (yes vs no)
Alcohol consumption during pregnancy (yes vs no)
Shoreline fish intake (≥once vs <once/week)
**
**
**
**
*
*
-0.20
-0.10
0.00
0.10
0.20
0.30
0.40
PCDDs/PCDFs and DL-PCBs (TEQ pg/g lipid)


Parity (≥1 vs 0)
**
Smoking history (yes vs no)
**
Blood sampling period (28 to <36 weeks vs <28 weeks)
Blood sampling period (≥36 weeks vs <28 weeks)
**
Blood sampling period (After delivery vs <28 weeks)
**
P for trend
0.000
Pelagic fish intake (≥once vs <once/week)
-0.35 -0.30 -0.25 -0.20 -0.15 -0.10 -0.05 0.00 0.05 0.10 0.15
PFOS (ng/mL)


Parity (≥1 vs 0)
**
Blood sampling period (28 to <36 weeks vs <28 weeks)
Blood sampling period (≥36 weeks vs <28 weeks)
*
Blood sampling period (After delivery vs <28 weeks)
*
P for trend
0.003
-0.30
-0.25
-0.20
-0.15
-0.10
-0.05
0.00
0.05
0.10
PFOA (ng/mL)


Fish intake (Quartile 2 vs Quartile 1)
Fish intake (Quartile 3 vs Quartile 1)
Fish intake (Quartile 4 vs Quartile 1)
P for trend
0.000
**
Pelagic fish intake (≥once vs <once/week)
*
Household income (million yen) (≥5 vs <5)
*
-0.10
-0.05
0.00
0.05
0.10
0.15
0.20
0.25
Hair Hg (µg/g)


1.4
1.2
1
0.8
0.6
0.4
0.2
0
log10-transformed PFOS (ng/mL)
0
5
10
15
20
25
30
Duration of maternal tobacco smoking (years)