【転帰】①在胎週数②出生体重③後期流産・早産の有無④胎児発育不全の有無

後期流産は「妊娠12週以降22週未満の死産」，早産は「妊娠22週以降の生産」，胎児発育不全は「妊娠22週以降に分娩となった症例の中で，我が国の在胎週数別・性別・初経産別の出生時体格基準表(板橋ら2010)において10%タイル未満のもの(SGA)」とした。

ANOVAおよび$\chi^2$検定，Tukey HSD検定，t-検定を用い，$p<0.05$を有意水準とした。

（倫理面への配慮）

本研究は，北海道大学環境健康科学研究教育センターおよび北海道大学大学院医学研究科・医の倫理委員会の承認を得た。本研究によって得られた個人名及び個人データの漏洩については，データの管理保管に適切な保管場所を確保するなどの方法により行うとともに，研究者の道義的責任に基づいて個人データをいかなる形でも本研究の研究者以外の外部の者に触れられないように厳重に保管し，取り扱った。

## C．研究結果

(1) 母の基本的属性と母体血清葉酸濃度・流早産

母年齢が高いほど血清葉酸濃度が高い傾向が認められた(Table 1-1)。葉酸サプリメント摂取群では，血清葉酸濃度が有意に高値であった(Table 1-2)。喫煙群では，血清葉酸濃度は有意に低かった。また，年収が高いほど血清葉酸濃度は高い傾向がみられた(Table 1-3)。

(2) 母体血清葉酸濃度四分位別の転帰

後期流産率と早産率，在胎週数，出生体重，SGAの割合は，血清葉酸濃度で分けた4群間でいずれも有意差を認めなかった(Table 2)。

(3) 在胎週数と母体血清葉酸濃度

在胎週数で分けた4群間で，在胎週数37週以上の群が，在胎週数28-36週の群よりも血清葉酸濃度が低かった(Table 3)。葉酸欠乏症に相当する血清葉酸濃度を示した20例(0.39%)のうち，19例は満期産であり，出生体重は3132±321g(平均±標準偏差)であった。

## D．考察

海外からの報告には，妊娠初期の血清葉酸濃度が低いことと早産や胎児発育不全の関連を示唆したものが幾つかあるが，本研究の結果は両者の関連を示さなかった。我が国の早産率が低い水準であることや，妊婦の栄養摂取の特徴，妊娠管理が充実していることなどが，低い血清葉酸濃度が早産や胎児発育不全の危険因子とならないことの要因かもしれない。

本研究では，血清葉酸値は妊婦の担当医に報告しているが，介入すべき血清葉酸値の基準は統一されていないため，妊婦への対応は医師に任せられている。結果を知らせた後に，葉酸摂取が行われたかについて調査されていないが，葉酸値の低いことを指摘された妊婦が，葉酸を摂取したことが影響した可能性は否定できない。

## E．結論

妊娠初期(第1三半期)の母体血清葉酸濃度と後期流産・早産，在胎週数，胎児発育に関連があるかについて，前向きコーホート研究へ参加した5,075名の単胎妊婦で検討した。その結果，我が国の妊娠女性においては，妊娠初期の血清葉酸濃度は，これらの妊娠転帰と関連しないことが示唆された。

## F．研究発表

1.論文発表

なし

2.学会発表
なし

## G. 知的財産権の出願・登録状況
該当なし

Table 1-1. Relationships between demographic characteristics, serum folate levels, late abortion, and preterm birth

| | | Serum folate level (nmol/L) | Late abortion | Preterm delivery |
|---|---|---|---|---|
| Overall | (n=5075) | 18.4 ± 21.6 | 48 (1.0%) | 220 (4.3%) |
| Maternal age (years) | | | | |
| ≤19 | (n=46) | 13.2 ± 4.0 | 0 (0.0%) | 2 (4.4%) |
| 20–29 | (n=2370) | 17.2 ± 8.1 † | 18 (0.8%) | 90 (3.8%) |
| 30–39 | (n=2565) | 19.6 ± 29.2 | 27 (1.1%) | 124 (4.8%) |
| ≥40 | (n=94) | 19.5 ± 10.7 | 3 (3.2%) | 4 (4.3%) |
| p-value | | 0.0004 [b] | 0.0825 [a] | 0.3626 [a] |
| prepregnancy BMI (kg/m$^2$) | | | | |
| <18.5 | (n=899) | 17.8 ± 11.0 | 9 (1.0%) | 42 (4.7%) |
| 18.5–24.9 | (n=3528) | 18.8 ± 25.0 | 31 (0.9%) | 140 (4.0%) |
| ≥25.0 | (n=550) | 17.3 ± 8.2 | 7 (1.3%) | 37 (5.6%) |
| p-value | | 0.2082 [b] | 0.6611 [a] | 0.1617 [a] |
| Parity | | | | |
| 0 | (n=1075) | 18.2 ± 9.4 | 8 (0.7%) | 46 (4.3%) |
| ≥1 | (n=2570) | 18.3 ± 24.2 | 33 (1.3%) | 107 (4.2%) |
| p-value | | 0.8855 [b] | 0.1587 [a] | 0.8739 [a] |

a: $\chi$2-test, b: ANOVA

BMI: Body mass index

†: p=0.0005 vs. age 30–39 years

Table 1-2. Relationships between demographic characteristics, serum folate levels, late abortion, and preterm birth

| | | Serum folate level (nmol/L) | Late abortion | Preterm delivery |
|---|---|---|---|---|
| Infertility treatment | | | | |
| no | (n=4871) | 18.3 ± 21.9 | 45 (0.9%) | 208 (4.3%) |
| yes | (n=185) | 20.8 ± 11.1 | 3 (1.6%) | 10 (5.4%) |
| p-value | | 0.1246 [b] | 0.3367 [a] | 0.4556 [a] |
| Folate supplementation | | | | |
| no | (n=4499) | 16.9 ± 13.8 | 43 (1.0%) | 194 (4.3%) |
| after conception | (n=389) | 29.2 ± 59.2 ‡ | 4 (1.0%) | 19 (4.9%) |
| before conception | (n=129) | 36.0 ± 19.0 ¶ | 1 (0.8%) | 5 (3.9%) |
| p-value | | <0.0001 [b] | 0.9888 [a] | 0.4694 [a] |

a: $\chi$2-test, b: ANOVA

‡: p= 0.0089 vs. no folate supplementaion, ¶: p<0.0001 vs. no folate supplementaion and folate supplementaion after conception

Table 1-3. Relationships between demographic characteristics, serum folate levels, late abortion, and preterm birth

| | | Serum folate level (nmol/L) | Late abortion | Preterm delivery |
|---|---|---|---|---|
| Smoking | | | | |
| no | (n=2207) | 19.5 ± 26.3 | 18 (0.8%) | 99 (4.5%) |
| yes | (n=2820) | 17.7 ± 17.2 | 30 (1.1%) | 119 (4.2%) |
| p-value | | 0.0038 [b] | 0.3691 [a] | 0.646 [a] |
| Alcohol | | | | |
| no | (n=1866) | 17.8 ± 16.9 | 18 (1.0%) | 92 (5.0%) |
| yes | (n=3167) | 18.8 ± 18.0 | 30 (1.0%) | 125 (4.0%) |
| p-value | | 0.1339 [b] | 0.9512 [a] | 0.0971 [a] |
| Annual income (USD) | | | | |
| <36,585 | (n=889) | 17.2 ± 9.7 | 11 (1.2%) | 33 (3.7%) |
| 36,586–60,974 | (n=1927) | 18.4 ± 20.1 | 11 (0.6%) | 87 (4.5%) |
| 60,975–97,560 | (n=1092) | 19.7 ± 35.5 | 16 (1.5%) | 47 (4.3%) |
| >97,561 | (n=323) | 20.2 ± 9.7 | 4 (1.2%) | 13 (4.0%) |
| p-value | | 0.066 [b] | 0.0817 [a] | 0.7985 [a] |

a: $\chi^2$-test, b: ANOVA

USD: United States Dollar, Exchange rate: 1 USD/ 82.00 Japanese Yen

Table 2. Preterm birth, gestational week at delivery, and fetal growth according to serum folate levels

| Serum folate level (nmol/L) | | Late abortion Preterm delivery | Gestational age at delivery (wk) | Birthweight (g) | SGA (<10%tile) |
|---|---|---|---|---|---|
| Overall | (n=5075) | 268 (5.3%) | 38.5 ± 2.9 | 3020 ± 485 | 7.1% |
| ≤13.1 | (n=1244) | 71 (5.7%) | 38.5 ± 3.0 | 3007 ± 474 | 5.5% |
| 13.2–16.1 | (n=1303) | 72 (5.5%) | 38.5 ± 3.1 | 3008 ± 528 | 7.6% |
| 16.2–20.0 | (n=1273) | 60 (4.7%) | 38.7 ± 2.4 | 3042 ± 447 | 6.8% |
| ≥20.1 | (n=1255) | 65 (5.2%) | 38.5 ± 2.8 | 3021 ± 487 | 8.4% |
| p-value | | 0.6928† | 0.2531 ‡ | 0.2372 ‡ | 0.1135† |

†: $\chi^2$-test, ‡: ANOVA

SGA: small for gestational age

Table 3. Serum folate levels according to gestational week at delivery

| Gestatinal week at delivery (week) | | Serum folate level (nmol/L) |
|---|---|---|
| Overall | (n=5075) | 18.4 ± 21.6 |
| 14–21 | (n=48) | 17.3 ± 12.0 |
| 22–27 | (n=12) | 20.4 ± 10.9 |
| 28–36 | (n=208) | 22.3 ± 55.8 |
| ≥37 | (n=4807) | 18.2 ± 18.8 † |

†: $p<0.05$ vs. 28–36 weeks

# 出生時体格に影響を及ぼす妊婦の受動喫煙曝露
# (Prenatal secondhand smoke exposure is associated with infant birth size)

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研究要旨

The hazardous effect of tobacco smoke exposure during pregnancy is a major public health concern. Maternal smoking has been associated with adverse birth outcomes. We investigated the association between secondhand smoke (SHS) exposure and infant birth size. A prospective cohort of 2,792 non-smoking Japanese pregnant women who delivered singleton babies at hospitals within Hokkaido Prefecture from 2003 to 2007 was conducted. Information on maternal and infants' characteristics, and lifestyle behaviors were gathered through self-administered questionnaires as well as hospital and birth records. Biochemical analysis of plasma cotinine was carried out using the enzyme-linked immunosorbent assay (ELISA) technique. Overall, an average increase in prenatal SHS exposure by about 0.66ng/mL, i.e. from 25$^{th}$ percentile (0.15ng/mL) to 75$^{th}$ percentile (0.81ng/mL) resulted in a mean reduction in birth weight of 61.4g (95% CI:-100.5, -22.3; $p$=0.002), birth length of 0.6cm (95%CI:-0.9, -0.2; $p$=0.002) and birth head circumference of 0.6cm (95%CI:-1.0, -0.1; $p$=0.007). The corresponding exposure brought about a mean birth weight deficit of 70g (95%CI:-124.9, -15.2; $p$=0.012) and head circumference deficit of 0.5cm (95%CI: -1.0, -0.03; $p$=0.037) in male infants and a birth length decrease of 0.8cm (95%CI:-1.3, -0.2; $p$=0.011) in female infants. This study concluded that maternal tobacco smoke exposure during pregnancy through passive route has negative effects on infant birth size especially the males.

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## A．研究目的

Maternal active smoking is associated with adverse pregnancy and birth outcomes such as placenta previa, abruptio placentae, ectopic pregnancy, spontaneous abortion, preterm delivery, low birth weight and perinatal mortality. Results of few studies on the negative effects of secondhand smoke (SHS) exposure on

birth outcomes are not clearly defined. Lack of clarity is probably due to the evaluation of SHS exposure using only self-administered questionnaires, measurement of cotinine levels in body fluids during the early gestation or failure to control for gestational age, a major determinant of birth weight.

In order to improve the reliability and validity of assessments by self-reported questionnaires, the use of biomarkers have been recently introduced. Cotinine, a widely studied biomarker, is a main metabolite of nicotine which can be found in blood, saliva, hair or urine analysis of SHS-exposed nonsmoker. Given the high prevalence of smoking among the Japanese men and the reluctance of most husbands to quit smoking during their wives' pregnancy, nonsmoking pregnant women in Japan are at a risk of exposure to SHS especially at home. The impact of SHS exposure on the unborn fetus remains a significant public health concern as birth size of a newborn greatly determines its survival, perinatal morbidity and adulthood health conditions.

Gender-specific difference in the effect of environmental pollutants on birth outcomes has been recently reported but with inconsistent results. Birth weight of male infants was more negatively affected by prenatal toxic exposure such as smoking, lead and airborne fine particles than the females. Contrariwise, maternal smoking during pregnancy has higher significantly negative influence on the mean birth weight and risk of small for gestational age (SGA) in newborn girls than in boys. In this study, we investigated the effects of maternal exposure to SHS during pregnancy on birth size using plasma cotinine concentrations as a biomarker for nicotine exposure. Gender-specific difference in fetal growth reduction was also assessed.

B．研究方法

A total of 2972 Japanese pregnant women were recruited from 2003 to 2007 from the ongoing "Hokkaido study on Environment and Children's Health". Vital information were gathered from baseline self-administered questionnaires, infants' hospital birth records and post–partum self-administered questionnaires. Biochemical analysis of maternal blood specimens collected during the third trimester was carried out through enzyme-linked immunosorbent assay (ELISA) technique (Cosmic Corporation, Japan) with a limit of detection of 0.12ng/mL.

Briefly, the ELISA 96-well plates coated with a rabbit anti-cotinine-4-bovine-γ-globulin polyclonal antibody were first incubated with 1% bovine serum albumin (BSA) after which 25μl of blood plasma samples and 100μl horseradish peroxidase-labeled (HRP) cotinine were added. The mixture was left to incubate at 20-25$^0$C for 1 hour. Subsequent to three

washes with 1% BSA, peroxidase substrate, tetramethylbenzidine, and $H_2O_2$ were added (Kirkegaad & Perry Laboratories, Gaithersburg, MD). The mixture was re-incubated for 30 minutes in the dark at the same temperature and 100µl phosphoric acid was added to the wells to stop enzyme activity. The absorbance was read at a wavelength of 450nm using an ELISA reader ($E_{max}$; Molecular Devices, Sunnyvale, CA)

The main birth outcomes were birth weight, length and head circumference and their relationship with maternal and infants' characteristics were examined with the student's t-test, Spearman's correlation test and analysis of variance (ANOVA). Next, their associations with exposure were examined using univariate and multivariate regression models. The main exposure variable (plasma cotinine concentration) was categorized by quartiles (<0.16, 0.16 - 0.33, 0.34 - 0.80 and >0.80ng/mL). First, their crude effects were examined and later adjusted for confounding variables such as maternal age, pre-pregnancy maternal weight, height, educational level, annual household income, parity, infant gender and gestational age at delivery. The subjects with undetectable cotinine levels were assigned a value half the detection limit (0.06ng/mL). Another multiple linear regression model was used to elucidate the gender-specific difference in the effect of SHS exposure on infant birth size by stratifying with infant gender. All statistical analyses were performed using the Statistical Packages for Social Sciences (SPSS, Inc., Chicago, USA) software for Windows version 16.0.

（倫理面への配慮）

This study was conducted with the informed consent of all subjects and approved by the institutional ethical board for human gene and genome studies of the Hokkaido University Center for Environmental and Health Sciences and the Hokkaido University Graduate School of Medicine.

## C．研究結果

In our earlier study, cut-off points for distinguishing the smoking statuses were established. Thus, those women whose plasma cotinine levels suggested active smoking (>11.48ng/mL ) were excluded.

In the current study, 80.7% of the overall study population has detectable plasma cotinine levels (≥0.12ng/mL). Table 1 summarizes the maternal and infants' characteristics in relation to birth size. Maternal age ranged from 17 to 44 years, with a mean (±SD) of 30.2 ± 4.4 years. Mean (±SD) pre-pregnancy weight and mean (±SD) maternal body mass index (BMI) were 52.8 ±8.6 kg and 21.1 ± 3.2 kg/m$^2$ respectively. More mothers were

multi-parous (50.5%), less educated (83.3%), low-income earners (54.3%) and drank alcohol during pregnancy (61.3%). Mean (±SD) gestational age was 39.1 ± 1.1 weeks. The associations of birth size with maternal and infants' characteristics are also shown in Table 1. Maternal height, pre-pregnancy weight, BMI and gestational age positively correlated with birth weight, birth length and head circumference ($p$<0.001). Maternal age positively correlated with birth head circumference but negatively with birth length ($p$<0.001). First infants were smaller than subsequent babies ($p$<0.001). Male infants were bigger, taller and had bigger head circumference than the females at birth ($p$<0.001).

Table 2 shows the maternal and infants' characteristics in relation to plasma cotinine levels. Mothers with the highest cotinine levels are more likely to be significantly younger, nulliparous (51.5%), less educated (56.0%), earn less than 5 million yen per annum (70.4%), have husbands who smoke more than 20 cigarettes per day (5.8%) in comparison to those in the lower cotinine quartile groups.

Figure 1 shows the mean birth weight, birth length and head circumference in relation to cotinine quartile levels respectively. The mean (±SD) birth weight, birth length and head circumference of infants in the highest cotinine quartile (Q4) were 3058.8 ± 362.6g, 48.9 ± 4.1cm, and 32.9 ± 3.8cm respectively; and lower in comparison to those of lower quartile groups.

Table 3 shows the multivariate regression analysis of birth size and plasma cotinine concentrations. In the adjusted model, the known risk factors of birth size from previous literature (maternal age, maternal height, maternal pre-pregnancy weight, educational level, annual household income, number of cigarettes smoked by partner per day, parity, infant gender, gestational age and delivery mode (included for head circumference)) were included. When all infants were considered, an inverse relationship of cotinine levels with infant birth weight, length and head circumference in the adjusted model was observed. Mothers in the highest quartile (>0.80ng/mL) group gave birth to babies with significantly lower birth weight (61.4g; 95%CI:-100.5, -22.3; $p$=0.002), birth length (0.6cm; 95%CI:-0.9, -0.2; $p$=0.002) and head circumference (0.6cm; 95%CI:-1.0, -0.1; $p$=0.007) than those in the lowest quartile group (<0.16ng/mL). Significant deficits in the mean birth weight (-70g; 95%CI:-124.9, -15.2; $p$=0.012) at the highest cotinine level and in the birth head circumference both at the 2nd (-0.6cm, 95%CI:-1.1, -0.1, $p$=0.011) and 4th quartiles (-0.5cm, 95%CI:-1.0, -0.003, $p$=0.037) of male infants were observed. On the other hand, reduction in the birth length of female infants was associated with

highest SHS exposure (-0.8cm, 95%CI:-1.3, -0.2, $p$=0.011).

D．考察

The major strength of this study is the use of plasma cotinine measurements for assessing SHS exposure which reduces the possibility of misclassification bias. The current study found an association of high SHS exposure during pregnancy with reduced mean birth weight, birth length and birth head circumference, before and after adjusting for potential confounding factors. Our finding of an inverse relationship of plasma cotinine concentrations with birth outcomes confirms the results of two studies which observed the greatest effect of prenatal SHS exposure on birth weight at the highest cotinine levels. A decrease of 108g in the mean birth weight of infants with mothers in the upper tertile cotinine levels (>1ng/mL) in comparison with those of lower tertile (<0.5ng/mL) mothers (Haddow et al. 1988). Also, neonates of women with cotinine levels >1.7ng/mL were 85g smaller than those of lowest quintile range (Rebagliato et al. 1995).

In a study of 263 nonsmoking African-Americans and Dominican women, high prenatal exposure to PAHs was associated with lower birth weight and smaller head circumference (Perera et al. 2003). Nicotine exposure on pregnancy causes a transient reduction in uterine blood flow due to early morphological changes of the placenta, while carbon monoxide in tobacco smoke can result in hypoxemia. Consequently, the birth weight, birth length and head circumference of the fetus may be reduced. The fundamental mechanisms of tobacco smoke effects on fetal growth need more clarifications and may possibly be multi-factorial.

Reduction in head circumference at birth has been correlated with a lower intelligence quotient (IQ), cognitive impairment, school performance in childhood and schizophrenia.

Our study provided an evidence of a gender difference in the effect of SHS exposure during pregnancy, with the male fetuses appearing to be more vulnerable to the exposure than the female ones. Overall average increase in exposure to SHS by about 0.66ng/mL gave rise to birth weight reduction of 70g (95% CI:-124.9, -15.2) in male infants compared to 50g (95%CI:-105.9, 5.7) in female infants. There are conflicting findings in previous studies regarding the effect of gender-specific differences. While a greater negative effect of heavy smoking in girls than in boys has been reported in a study, male fetuses have been observed to be more susceptible to the negative effect of active tobacco smoke than the females in other studies (Zaren et al. 2000, Varvarigou et al. 2009). Prenatal

exposure to fine particulate matters affected the birth size of male infants more than females (Jedrychowski et al. 2009). The gender-specific effect can be explained by the fact that the growth rate of male fetuses is normally higher than the females, hence, the effect of the factors restraining fetal development could be greater in fetuses with faster intrauterine growth. Demands on blood circulation, oxygen or fuels related to faster growth rate are also greater by the male gender. Differences exist in hormonal conditions based on gender. During the second half of the second trimester of pregnancy, the vulnerability to toxic effects of tobacco smoke chemicals increases as the brain begins its growth spurt (for example, glial cells and axon growth, dendrites' branching and synapses' formation) at this period.

The design of this study as a prospective cohort reduces recall bias. Second, exclusion of preterm births eliminates confounding from pregnancy complications and concentrates on fetal development. Third, the selection of study participants from 37 hospitals within Hokkaido eradicates the possibility of selection bias.

## E．結論

SHS exposure obviously has adverse effects on fetal growth especially among the male fetuses. The greater vulnerability of the male fetuses may be due to their faster intrauterine growth and different hormonal conditions. It remains unclear why the birth length of the female fetuses suffers more negative effect than that of the males. There is the need to further investigate into this in future studies.

## F. 研究発表

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G．知的財産権の出願・登録状況
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Table 1. Maternal and infants' characteristics in relation to birth outcomes (N= 2972)

| Variables | No. | Birth weight (g) | *p* value | Birth length (cm) | *p* value | Birth head circumference (cm) | *p* value[a] |
|---|---|---|---|---|---|---|---|
| Maternal age (years) | | r = -0.025 | 0.178 | r = -0.042 | 0.023 | r = 0.058 | 0.002 |
| Maternal height (cm) | | r = 0.173 | <0.001 | r = 0.188 | <0.001 | r = 0.154 | <0.001 |
| Pre-pregnancy maternal weight (kg) | | r = 0.219 | <0.001 | r = 0.183 | <0.001 | r = 0.177 | <0.001 |
| Maternal BMI (kg/m$^2$) | | r = 0.153 | <0.001 | r = 0.105 | <0.001 | r = 0.114 | <0.001 |
| Parity | | | | | | | |
| Nulliparous | 1361 | 3078.0 ± 354.3 | 0.001 | 49.2 (2.3) | 0.046 | 33.0 (3.1) | 0.314 |
| Multiparous | 1501 | 3124.8 ± 365.5 | | 49.0 (3.6) | | 33.1 (3.7) | |
| Alcohol intake during pregnancy | | | | | | | |
| No | 1126 | 3102.5 ± 359.8 | 0.725 | 49.0 (3.7) | 0.080 | 33.0 (3.9) | 0.345 |
| Yes | 1823 | 3097.7 ± 361.1 | | 49.2 (2.8) | | 33.1 (3.2) | |
| Educational level (years) | | | | | | | |
| ≤12 | 2477 | 3100.0 ± 359.1 | 0.809 | 49.2 (2.3) | 0.031* | 33.1 (3.1) | 0.261 |
| >12 | 492 | 3096.1 ± 366.9 | | 48.1 (3.7) | | 33.0 (3.8) | |
| Annual household income (millions of Yen) | | | | | | | |
| <5 | 1615 | 3102.0 ± 362.3 | 0.736 | 49.1 (3.1) | 0.246 | 33.0 (3.3) | 0.780 |
| ≥5 | 888 | 3096.1 ± 366.9 | | 49.0 (3.4) | | 33.1 (3.9) | |
| Number of cigarettes smoked by partner per day | | | | | | | |
| 0 | 1457 | 3101.0 ± 373.9 | 0.998 | 49.1 (3.2) | 0.500 | 33.0 (3.3) | 0.834 |
| 1 – 9 | 427 | 3104.6 ± 368.0 | | 49.1 (3.0) | | 33.0 (4.0) | |
| 10 – 19 | 526 | 3103.4 ± 333.8 | | 48.9 (4.1) | | 33.1 (3.3) | |
| ≥20 | 539 | 3102.8 ± 380.0 | | 49.2 (1.9) | | 33.2 (3.3) | |
| Gestational age (weeks) | | r = 0.303 | <0.001 | r = 0.316 | <0.001 | r = 0.112 | <0.001 |
| Infant gender | | | | | | | |
| Male | 1436 | 3146.9 ± 354.9 | <0.001 | 49.5 (2.9) | <0.001 | 33.3 (3.2) | <0.001 |
| Female | 1536 | 3056.2 ± 360.1 | | 48.8 (3.4) | | 32.8 (3.7) | |

[a] Spearman's correlation test, Student's t test, ANOVA
[b] Mean (SD)
BMI, body mass index SD, standard deviation

Table 2. Maternal characteristics in relation to plasma cotinine levels during pregnancy (N= 2972)

| Variable | <0.16ng/mL (n = 784) | 0.16 - 0.33ng/mL (n = 714) | 0.34 - 0.80ng/mL (n = 743) | >0.80ng/mL (n = 731) | *p* value[a] |
|---|---|---|---|---|---|
| Maternal age (years)[b] | 31.1 (4.2) | 30.6 (4.1) | 29.8 (4.2) | 29.1 (4.5) | <0.001⋕ |
| Nulliparous | 336 (43.6) | 339 (48.8) | 339 (46.9) | 347 (51.5) | 0.024 |
| Multiparous | 434 (56.4) | 356 (51.2) | 384 (53.1) | 327 (48.5) | |
| Alcohol intake during pregnancy | | | | | |
| No | 292 (37.5) | 269 (38.0) | 287 (39.0) | 278 (38.2) | 0.939 |
| Yes | 487 (62.5) | 439 (62.0) | 448 (61.0) | 449 (61.8) | |
| Educational level (years) | | | | | |
| ≤12 | 277 (35.7) | 294 (41.4) | 326 (44.2) | 405 (56.0) | <0.001 |
| >12 | 498 (64.3) | 416 (58.6) | 412 (55.8) | 318 (44.0) | |
| Annual household income (millions of Yen) | | | | | |
| <5 | 390 (59.4) | 378 (61.2) | 427 (67.7) | 420 (70.4) | <0.001 |
| ≥5 | 267 (40.6) | 240 (38.8) | 204 (32.3) | 177 (29.6) | |
| Number of cigarettes smoked by partner per day | | | | | |
| 0 | 565 (72.5) | 385 (54.1) | 308 (41.6) | 203 (27.8) | <0.001 |
| 1 – 9 | 119 (15.3) | 188 (26.4) | 216 (29.2) | 257 (35.3) | |
| 10 –19 | 90 (11.6) | 122 (17.1) | 179 (24.2) | 227 (31.1) | |
| ≥20 | 5 (0.6) | 17 (2.4) | 37 (5.0) | 42 (5.8) | |

[a]Chi square test, ANOVA ⋕Kruskal Wallis test
[b]Mean (SD)

Table 3. Association of cotinine concentrations in maternal plasma with infant birth size (N = 2972)

| | Birth weight (g) | | Birth length (cm) | | Head circumference (cm) | |
|---|---|---|---|---|---|---|
| | β (95%CI | *p* value | β (95%CI | *p* value | β (95%CI | *p* value |
| **All infants (N = 2972)** | | | | | | |
| **SHS exposure** | | | | | | |
| <0.16ng/mL | Reference | | Reference | | Reference | |
| 0.16 – 0.33ng/mL | -23.7 (-61.5, 14.1) | 0.797 | -0.1 (-0.5, 0.2) | 0.542 | -0.3 (-0.7, 0.1) | 0.145 |
| 0.34 – 0.80ng/mL | -32.6 (-70.4, 5.7) | 0.154 | -0.2 (-0.5, 0.2) | 0.353 | -0.3 (-0.6, 0.1) | 0.198 |
| >0.80ng/mL | -61.4 (-100.5, -22.3) | 0.002 | -0.6 (-1.9, -0.2) | 0.002 | -0.6 (-1.0, -0.1) | 0.007 |
| **Male infants (n = 1436)** | | | | | | |
| **SHS exposure** | | | | | | |
| <0.16ng/mL | Reference | | Reference | | Reference | |
| 0.16 – 0.33ng/mL | -7.0 (-59.9, 46.0) | 0.797 | -0.2 (-0.6, 0.2) | 0.352 | -0.6 (-1.1, -0.1) | 0.011 |
| 0.34 – 0.80ng/mL | -38.6 (-91.7, 14.4) | 0.154 | -0.3 (-0.7, 0.1) | 0.166 | -0.4 (-0.9, 0.1) | 0.121 |
| >0.80ng/mL | -70.0 (-124.9, -15.2) | 0.012 | -0.4 (-0.9, 0.1) | 0.093 | -0.5 (-1.0, -0.03) | 0.037 |
| **Female infants (n = 1536)** | | | | | | |
| **SHS exposure** | | | | | | |
| <0.16ng/mL | Reference | | Reference | | Reference | |
| 0.16 – 0.33ng/mL | -35.2 (-88.6, 18.2) | 0.191 | -0.04 (-0.6, 0.5) | 0.925 | -0.02 (-0.6, 0.6) | 0.962 |
| 0.34 – 0.80ng/mL | -27.7 (-81.2, 25.9) | 0.331 | -0.1 (-0.6, 0.5) | 0.840 | -0.1 (-0.8, 0.5) | 0.654 |
| >0.80ng/mL | -50.1 (-105.9, 5.7) | 0.092 | -0.8 (-1.3, -0.2) | 0.011 | -0.6 (-1.2, 0.04) | 0.079 |

CI, confidence interval

β: Change in birth weight after adjusting for maternal age, height, pre-pregnancy maternal weight, educational level, annual household income, parity, infant gender and gestational age.

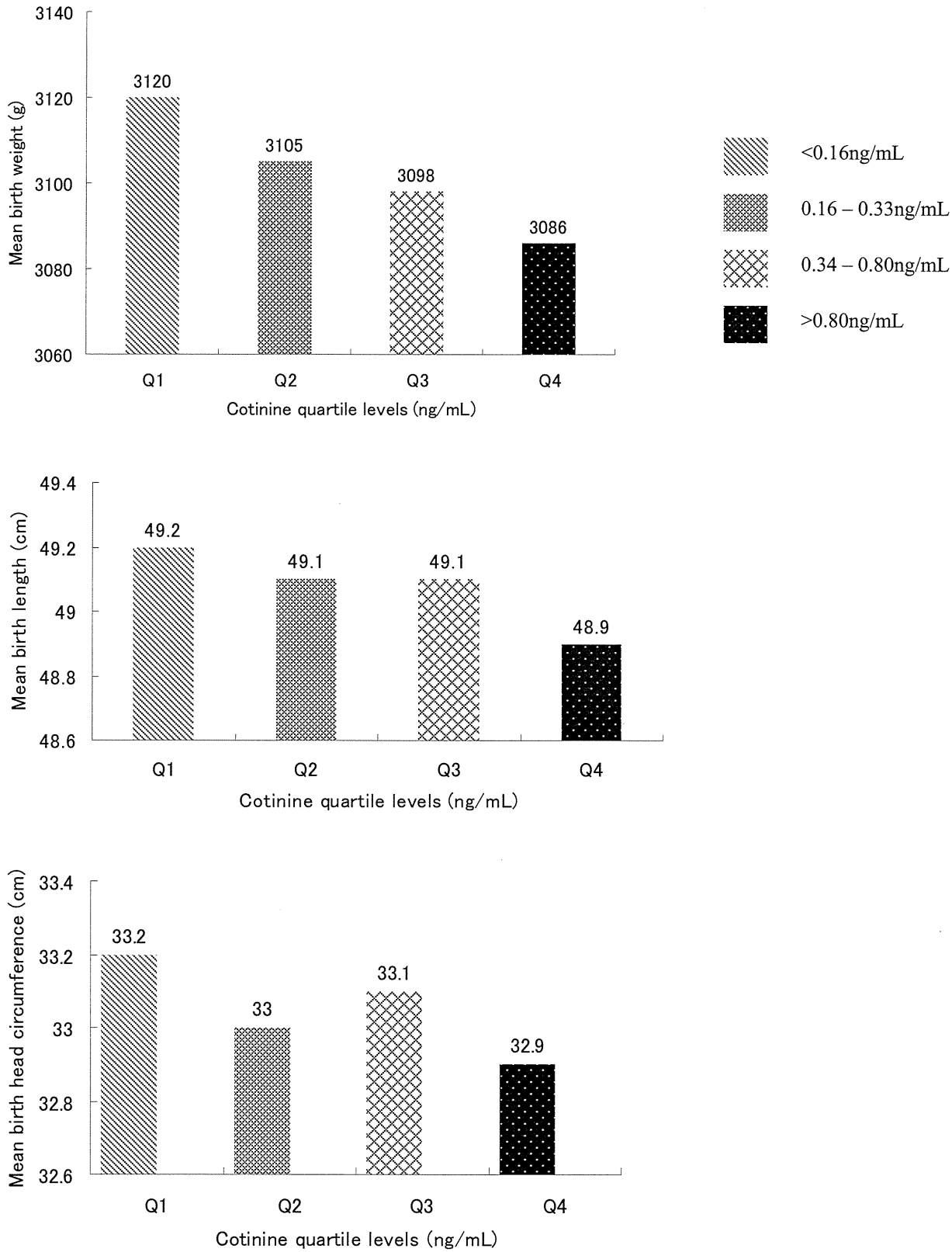


Figure 1: Fetal growth parameters and SHS exposure levels

# 母体血液中の PCBs・ダイオキシン類，有機フッ素化合物および毛髪水銀濃度に関連する要因の基礎的検討

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**研究要旨**

PCBs・ダイオキシン類，有機フッ素化合物，水銀は生体から検出される環境化学物質である。これら物質は妊婦から胎児へ移行して，その発育を阻害する可能性がある(Toft et al., 2004)。体内の曝露量に影響する要因は国や地域によって異なるが，わが国の妊婦の曝露量と生活・食習慣などの多様な要因を検討した報告はわずかである。本研究では，一般生活環境の母体血および毛髪中の環境化学物質濃度に関連する要因を検討した。対象者は一般病院・産科を受診した妊娠 23 週～35 週の妊婦で，妊婦とその配偶者から，自記式調査票により既往歴，教育歴，世帯収入，ライフスタイルなどの情報を得た。妊娠後期の母体血で 426 名の PCBs・ダイオキシン類濃度(HRGC/HRMS 法)および 447 名の PFOS，PFOA 濃度(LC/MS/MS 法)，さらに分娩直後の毛髪で 430 名の総水銀濃度(AAS 法)を測定した。重回帰分析で，各濃度(log10)を従属変数，関連要因を独立変数として，環境化学物質に有意に影響する要因を検討した。交絡要因として母親の属性(年齢，出産歴など)，両親の喫煙・飲酒状況，分娩年，採血時期，および食物摂取頻度などで調整した。重回帰分析では，PFOA を除く環境化学物質濃度は分娩年経過と共に低下し，環境中の曝露レベルの低下を反映していると考えられた。妊娠前，妊娠中の喫煙は妊婦のダイオキシン類と PFOS 濃度を低下させ，喫煙による酵素誘導が胎児・胎盤を含む母体外への排出を亢進させると考えられた。妊娠中の飲酒，魚摂取量，牛肉摂取により母体血中濃度が増加したことから，食生活の欧米化が曝露源の変化や飲酒による肝代謝変化が関与すると考えられた。

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## A．研究目的

PCBs・ダイオキシン類，有機フッ素化合物・水銀は生体から広く検出される環境化学物質である。これら物質の主な曝露源は食物であり，日本では海産物による曝露

の割合が大きいと報告されている(Geary 2007; Todaka 2010; Murata 2011)。体内の曝露量は，食生活，年齢，体脂肪，喫煙，飲酒，生活環境などの多様な要因と相互的に影響するため，曝露量に関連する要因は国や地域で異なる。

出産可能年齢の女性を含む日本の成人1656名では，魚介類やアルコール摂取が増加するほど血中 PCBs・ダイオキシン類が有意に増加した(Arisawa 2011)。海外では，妊婦の曝露量に関連する要因として，飲酒や社会経済的要因を検討している。わが国の妊婦を対象とした東北と東京の研究では，複数の要因を調整する多変量解析により，母体血，臍帯血および母乳中 PCBs・ダイオキシン類が出産歴，母親の年齢および喫煙歴と有意に関連することを示した(Tajimi et al., 2006; Nakamura et al., 2007)。

しかし，一般環境における日本人妊婦の有機フッ素化合物(PFOS, PFOA)濃度については報告がまだなく，また，低濃度の水銀に関しては，東北地域での研究が1報あるが，対象者数が少ないため，社会経済的要因や食生活の影響に関する検討が十分に行われていない。

そこで本研究では，一般生活環境における妊婦の環境化学物質濃度に関連する要因のプロファイリングを飲酒や社会経済的な要因を含めて検討することを目的とした。

## B．研究方法

対象者は2002年7月から2005年10月に札幌市の一般病院・産科を受診した妊娠23週～35週の妊婦で，インフォームドコンセントを得られた母児514組である。妊婦とその配偶者から，自記式調査票により既往歴，教育歴，世帯収入，ライフスタイルなどの情報を得た。妊娠中期～後期に母親から採血し，高分解能ガスクロマトグラフィー・高分解能マススペクトメトリー法(HRGC/HRMS 法)で，426名の母体血中 PCBs・ダイオキシン類濃度，およびオンライン国相抽出—高速液体クロマトグラフィタンデム質量分析法(LC/MS/MS 法)で447名の母体血中 PFOS，PFOA 濃度を測定した。分娩後5日以内に採取した430名の母親の毛髪中総水銀濃度(Hg)を酸化燃焼金アマルガム法(AAS 法)で測定した。PCBs・ダイオキシン類を測定した426名のうち1名は PCDFs が異常高値を示したので，解析から除外した。

環境化学物質の濃度と両親の属性，飲酒，喫煙，社会経済的要因および妊婦の食事摂取頻度などの項目との関係は，Spearman の相関係数および Student's の t 検定を用いて解析した。各濃度の相関は Spearman の相関係数を用いて解析した。対数変換した各物質濃度を従属変数に，全ての関連要因を独立変数として投入し，重回帰分析のステップワイズ法により各物質濃度に有意に関連する要因を選択した。投入した変数は母親の属性(年齢，出産歴など)，両親の喫煙・飲酒状況，分娩年，採血時期，食物摂取頻度，および魚摂取量等である。毛髪水銀濃度の解析にのみ母親のパーマ状況を追加で投入した。また，PCBs・ダイオキシン類および PFOA，PFOS 濃度の解析にのみに採血時期(出産前，出産後)を追加投入した。

（倫理面への配慮）

北海道大学環境健康科学研究教育センターおよび北海道大学大学院医学研究科医の倫理委員会および研究協力施設の研究倫理委員会に諮り，承認を得たうえで実施した。

## C．研究結果

環境化学物質の濃度(中央値)はそれぞれ PCBs=107 ng/g lipid, Total ダイオキシン

類 =13.9 TEQ pg/g lipid , PFOS=5.20ng/ml, PFOA=1.30ng/ml, および Hg=1.40ppm であった（表 1)。それぞれの物質濃度の相関は，PCBs とダイオキシン類が最も強く(r=0.808, p<0.001), PCBs と Hg (r=0.380, p<0.001), ダイオキシン類と Hg (r=0.318, p<0.001), ダイオキシン類と PFOS (r=0.257, p<0.001) および PFOS と PFOA(r=0.240, p<0.001) で中程度の相関が認められた(表 2)。単変量解析では，各物質濃度と母親の年齢，身長，出産歴，採血時期，分娩年，世帯収入，北海道の居住期間，母親の喫煙歴，妊娠中飲酒，1 日魚摂取量，遠海魚摂取，および牛乳摂取が有意に関連した(表 3)。ステップワイズ法で選択した母親の年齢，非妊娠時体重，出産歴，分娩年，採血時期，魚推定摂取量，遠海魚摂取頻度，および牛肉摂取頻度を重回帰分析の最終モデルとした(表 4)。

各物質濃度は，母親の年齢，非妊娠時体重，母親の妊娠中飲酒，魚推定摂取量(g/day)，遠海魚摂取頻度および牛肉摂取頻度と正の関連を示した。特に，母親年齢の PCBs およびダイオキシン類濃度に対する標準化回帰係数(Std β)，分娩年の PFOA 濃度に対する標準化回帰係数は正の関連を示す変数の中で高かった(PCBs:Std β=0.469, 95%CI ダイオキシン類:Std β=0.348, PFOA:Std β=0.204)。

一方で，各物質濃度は，母親の出産歴，母親の喫煙歴，出産後採血，北海道の居住期間と負の関連を示した。特に母親出産歴の PCBs・ダイオキシン類と PFOA 濃度に対する標準化回帰係数 (PCBs:Std β=-0.292, ダイオキシン類:Std β=-0.359, PFOA:Std β=-0.379)，分娩年のダイオキシン類および PFOS 濃度に対する標準化回帰係数(ダイオキシン類:Std β=-0.206, PFOS:Std β=-0.455)，測定時期の PFOS 濃度に対する標準化回帰係数(PFOS:Std β=-0.226)は負の関連を示した変数の中で高かった。分娩年と PFOA を除く物質濃度は負の関連を示した。

さらに，魚推定摂取量と遠洋魚の総水銀濃度(魚摂取量/Hg:Std β=0.185, 遠洋魚/Hg:Std β=0.149)および牛肉摂取量の PCBs 濃度(牛肉摂取量/PCBs:Std β=0.090)に対する標準化回帰係数は正の関連を示した。

## D．考察

本研究から，妊婦の母体血および毛髪中の環境化学物質濃度は母親の年齢，非妊娠時体重，出産歴，分娩年，北海道居住期間，喫煙歴，妊娠中飲酒状況，1 日魚推定摂取量，遠海魚摂取頻度および牛肉摂取頻度と有意に関連することが示された。

本研究ならびにわが国における先行研究では，母親の加齢と共に PCBs・ダイオキシン類は増加していたことから (Nakamura et al., 2008; Tajimi et al., 2006)，一般生活環境による日常の摂取量が排出量を上回り，結果的に加齢とともに蓄積量が増加することが示唆された。

本研究では，母体血中 PCBs・ダイオキシン類および PFOS・PFOA 濃度は初産妊婦より経産妊婦で低かった。妊婦を対象にした先行研究でも同様の結果が認められており，女性に特有となる PCBs・ダイオキシン類の排出経路が妊娠から出産後の時期に存在すること示した日本および諸外国の先行研究と一致した(Milbrath et al., 2008; Nakamura et al., 2007; Tajimi et al., 2006)。

環境モニタリングや疫学研究では，PCBs・ダイオキシン類の環境中レベルが経年的に減少していることが報告されており(Link et al., 2007)，本研究でも認められた母親の加齢に伴う PCBs・ダイオキシン類濃度の増加および分娩年経過に伴う減少