Evidence that birth weight is decreased by lead at maternal blood levels below 5 μg/dl in male but not in female newborns

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Abstract

To assess the association between birth weight and maternal blood lead (BPb) levels, 386 pregnant women and their newborn offspring were surveyed. Mean ± SD (range) maternal BPb concentrations were 0.98 ± 0.55 (0.10–3.99), 0.92 ± 0.63 [<0.09 (limit of quantification) - 3.96], and 0.99 ± 0.66 (<0.09–3.96) μg/dl at 12, 25 and 36 weeks' gestation, respectively. Mean ± SD (range) gestational age at delivery was 38.9 ± 1.3 (35–41) weeks. In male newborns, a significant correlation between birth weight and logBPb at 12 weeks' gestation was observed (Spearman's rank correlation coefficient = - 0.145, $p < 0.05$). Multiple regression analysis indicated that birth weight was significantly inversely associated with logBPb at 12 weeks' gestation, controlling for possible confounding variables. These results suggest that low-level exposure to lead in early gestation could be a risk factor for reduced birth weight in male offspring.

Keywords: Lead, birth weight, prenatal exposure, pregnancy outcome, sex differences

1. Introduction

Pregnancy is a unique period of a woman's life, in which there is a high sensitivity to toxic substances [1-6]. Both chronic and acute exposure to lead can increase blood lead (BPb) levels during pregnancy [7-9], and lead can freely cross the placenta [10-13]. Several studies have reported a positive association between maternal BPb and the risk of spontaneous abortion [14-15], preterm birth [16-17], pregnancy-induced hypertension [18-26], and premature rupture of the membranes [27]. These observations were reported in groups of pregnant women with relatively low BPb, i.e. maximum level 6.5–24.6 μg/dl, mean level 0.66–10.1 μg/dl.

In addition to these findings, the effects of lead on birth weight may represent an important medical problem, because low birth weight is an important predictor of neonatal mortality and morbidity [28-29]. However, previous epidemiologic studies on the association between maternal BPb and birth weight have yielded varied results. Significant associations were observed at a maternal BPb of 1.0–26.0 μg/dl [30], 1.0–11.9 μg/dl [31], 10 μg/dl or above [32], and below 10 μg/dl [33]. Furthermore, small-for-gestational age and intrauterine growth retardation were reported to be related to umbilical cord BPb of 0.1–35.1 μg/dl [34]. In contrast, no significant associations were found between birth weight and maternal BPb at 0.08–2.64 μmol/l (1.65–54.6 μg/dl) [35] or at a mean concentration of 10.6 μg/dl [36]. Recently, BPb levels have been reported to be very low in pregnant women without occupational exposure, e.g. ranging from <0.09 to 4.03 μg/dl in pregnant women in Japan [37]. This meets the current guidelines from the Centers for Disease Control and Prevention (CDC) that BPb should be below 5 μg/dl for women of childbearing age and for children [38]. The major purpose of the present study was to establish if birth weight is affected by lead at a maternal level of below 5 μg/dl.

During the course of normal pregnancy, changes in maternal BPb have been observed:

A study in Mexico City demonstrated a decrease (1.1 μg/dl) in mean BPb from week 12 to week 20 of gestation and an increase (1.6 μg/dl) in mean BPb from week 20 to parturition in 105 women with a mean BPb of 7.0 μg/dl (range 1.0–35.5 μg/dl) [39]. Such U-shaped changes in BPb during pregnancy were also observed in 12 women in Sydney whose BPb was less than 6.5 μg/dl [40], and in 195 women in Pittsburgh [41] (BPb not reported). These changes are possibly due to changes in gastrointestinal absorption and bone storage of lead during pregnancy [9], although they are not fully understood. It has also been suggested that lead in maternal blood can readily cross the placenta and enter fetal blood circulation from the 12th to the 14th week of gestation [10]. Thus, in an attempt to establish the critical time point of reproductive toxicity of lead, blood samples were collected during the first, second and third trimesters of pregnancy in the present study.

Several previous studies have suggested sex differences in lead toxicity in humans. Lead-induced hypertension is observed more often in males than in females [42]. Neuropsychological development is more often impaired in male children than in females [43-45]. In addition, we have observed that BPb is higher in male children [50]. Experimental animal studies have also demonstrated sex differences in the effects of lead. Lead-induced hypertension and motor deficits are observed only in male mice [46, 47]. In contrast, depressive-like behavior and delayed-type immune hypersensitivity are caused by lead exposure in female animals [48, 49]. Sex-related differences in the effects of lead on birth weight was therefore also considered in the present study.

2. Subjects and Methods

2.1. Subjects

The study was conducted on pregnant women from early gestation (week 12) who visited Juntendo University Hospital, Tokyo, Japan from December 2010 to October 2012. During this period, a total of 602 pregnant women who met the criteria for the study (i.e., women aged ≥ 20 years, had a singleton pregnancy and were scheduled for delivery at the University Hospital) visited the hospital for regular checkups. Of these, we were able to communicate with 582 women, and 540 agreed to participate in the study. The study excluded women who had spontaneous and/or induced abortion (n = 12), intra-uterine fetal death (n = 2), essential hypertension (n = 2), a heart pacemaker (n = 1), congenital biliary atresia (n = 1) or breast cancer during the current pregnancy (n = 1). During the first trimester, we were unable to collect blood samples from fifteen pregnant women because they had been referred from other hospitals for regular checkups. Thus, a total of 506 pregnant women was followed until delivery (i.e., from December 2010 to May 2013). One-hundred and twenty subjects were excluded from the analysis because blood samples were not obtained from them at all 3 times during the study. The final analysis thus included 386 subjects. There were no significant differences in general characteristics (Table 1) between these 386 women and the 120 women excluded from the study.

2.2. Collection and analysis of blood samples

BPb was measured as reported in our previous studies [16, 23-24, 27, 37, 50]. Venous blood samples were collected at 12, 25 and 36 weeks during pregnancy from the cubital vein using vacuum tubes (Venoject VP-H070K, Terumo, Tokyo, Japan). Samples were frozen at –80 °C until measurement. Blood samples (in 0.1 ml volumes) were put into a

perfluoroalkoxy teflon bottle, then 0.4 ml of concentrated nitric acid (Ultrapure Grade, Tama Chemicals Co., Kawasaki, Japan) was added and the samples left overnight. The sample mixture was digested with 0.2 ml hydrogen peroxide (Ultrapure Grade, Tama Chemicals Co., Kawasaki, Japan) in a microwave oven (MLS-1200 MEGA, Milestone S.R.L., Bergamo, Italy) in five steps with power set at 250, 0, 250, 400 and 600 W for 5, 1, 5, 5, and 5 min, respectively; the volume of the digested sample was then adjusted to 1.0 ml with ultrapure water. After dilution with 0.5% nitric acid, lead concentrations were measured using an inductively coupled plasma mass spectrometer (ICP-MS, Eran DRC-II, PerkinElmer, Waltham, MA, USA) at mass-to-charge ratio (m/z) 208 using an external standard multi-element standard (XSTC-13 SPEX CertiPrep. Inc., Metuchen, NJ, USA). The BPb measurements were repeated three times and the average was used for subsequent analysis. For instrument calibration throughout the measurements, at least 10% of the analyses were of the external standard, and 5% were of a blank (pure water).

2.3. Quality control and quality assurance

Accuracy of BPb measurement was assessed using Certified Reference Materials (CRMs), specificially BCR 634, BCR 635, and BCR 636 provided by the Institute for Reference Materials and Measurements, European Union. The certified values of lead in BCR 634, BCR 635, and BCR 636 were 46, 210, and 520 µg/dl, respectively. CRMs were used as accuracy control samples and their lead concentrations were measured by the same method as the blood samples taken from study subjects. Averages of measured values obtained from eight samples each for BCR 634, BCR 635, and BCR 636 were 47, 209 and, 526 µg/dl, respectively, with relative standard deviations of 2.9, 2.5, and 2.6%, respectively. All results obtained for CRMs were in agreement with certified values at the 95% confidence level using

Student's t-test.

The limit of detection (LOD) and the limit of quantification (LOQ) were the concentration equivalent to the signal of lead, which was equal to three and 10 times the standard deviation of 10 repeated measurements of the blank signal at m/z 208, respectively. The values of LOD and LOQ were 0.03 and 0.09 μg/dl, respectively. In the present study, measured values lower than LOQ (6 samples) were replaced by half the LOQ for the analysis, as in a previous study [51].

2.4. Questionnaires and medical information

The study participants completed a structured questionnaire at 12 weeks' gestation. The questions were focused on socio-demographic and lifestyle information, such as alcohol consumption both before and during pregnancy, and smoking (number of cigarettes/day). Data regarding potential confounding factors were collected, e.g., maternal age, level of education, and annual household income. Information regarding the history of any illness and maternal physical information during pregnancy was recorded when the pregnant women came to the obstetric clinic of the university hospital for regular checkups. Mode of delivery (Caesarian section *vs.* vaginal delivery) and birth weight were obtained from the delivery records. All newborns' weights were measured immediately after birth (i.e., within 5 minutes after the birth). Maternal height and weight immediately prior to delivery were measured at admission for delivery.

2.5. Statistical analysis

To reduce the influence of outliers and to normalize the residual distribution, the common logarithm of BPb concentration was used in the statistical analysis. One-way repeated-measures analysis of variance was used to examine differences between the maternal BPb at 12, 25 and 36 weeks of pregnancy. A Mann-Whitney U-test was used to analyze differences in maternal BPb between male and female newborns. Spearman's rank correlation coefficient was calculated to assess associations between birth weight and BPb. Multiple regression analysis was used to examine the relationships between BPb and birth weight, controlling for possible confounding variables such as hematocrit, maternal age, maternal body mass index (BMI), gestational age and alcohol consumption. The statistical analyses were conducted using the Statistical Package for the Social Sciences 20 (IBM, Japan Inc., Tokyo, Japan). Data are presented as mean ± standard deviation (range) unless otherwise stated.

2.6. Ethical aspects

The present study was conducted under the approval of the Ethics Committee of Juntendo University Hospital (authorization number 632). Written informed consent was obtained from all participants after explaining the purpose and procedures of the study, privacy protection, and the right to withdraw from the study whenever they wished.

3. Results

Characteristics of the 386 mothers included in the final analysis are shown in Table 1. At the three measurement times, mean maternal BPb was less than 1.0 μg/dl and the

maximum was below 4 μg/dl. Although not statistically significant ($p > 0.05$), maternal BPb was decreased at 25 weeks and then increased at 36 weeks of gestation, indicating the U-shaped change during the pregnancy. No statistical differences in maternal BPb were observed between male and female newborns (p = 0.363–0.839). Mean birth weight was 3125.5 ± 362.9 (2212–4518) g for males and 2993.4 ± 388. 9 (2032–4314) g for females. Gestational age was 38.9 ± 1.2 (35–41) weeks for males and 38.9 ± 1.3 (35 - 41) weeks for females. Birth weight was significantly different between males and females (p = 0.001), whereas no significant sex differences were observed in gestational age (p = 0.755). A significant correlation between birth weight and BPb at 12 weeks' gestation was observed in male newborns (Figure 1). The results of multiple regression analysis showed that, in male newborns, birth weight was significantly related to logBPb at 12 weeks' gestation, as well as to gestational age (Table 2). The significant relationships of birth weight to logBPb at 12 weeks' gestation and to gestational age were also observed when five mothers who smoked during pregnancy were excluded from the multiple regression analysis, as well as when "drinking before pregnancy" was used as the independent variable instead of "drinking during pregnancy." In female newborns, no significant relationships were found between birth weight and maternal BPb concentration (rs = -0.087, p = 0.234). Figure 2 shows the relationship between birth weight and logBPb, adjusting for the confounding variables listed in Table 2.

4. Discussion

The present study shows a significant relationship between maternal BPb at 12 weeks' gestation and birth weight in male offspring. This was confirmed by multiple regression analysis, adjusting for possible confounding variables. Thus, lead may decrease birth weight in males at a maternal BPb below 5 μg/dl, which was measured at an early stage of gestation.

In contrast, no significant relationships were observed between maternal BPb and birth weight of female newborns, suggesting sex differences in the effects of lead.

Because maternal BPb can readily cross the placenta and enter fetal blood circulation from 12–14 weeks' gestation [10], *in utero* exposure to lead at an early stage of gestation seems a serious risk factor for reduced birth weight. It could be hypothesized that lead impairs normal fetal bone growth by interfering with the deposition of calcium into bone, as suggested by the reviews of Potula et al. [52] and Pounds et al. [53]. Further studies are necessary to clarify whether such effects underly the adverse effects of very low level lead on birth weight as observed in the present study. Maternal BPb levels in the present study were lower than those in previous studies which showed a significant association between maternal BPb and decreased birth weight [30-34], as well as those in two studies which failed to reveal such an association [35, 36]. However these studies did not refer to the sex of newborns; taking this into account seems be important to correctly evaluate the effects of lead on birth weight.

As reviewed by Llop et al. [43], neurotoxic effects of prenatal lead exposure seem to be more pronounced in male children [44, 45], although one study failed to demonstrate this [53]. Similarly, experimental animal studies have shown that prenatal and postnatal exposure to lead result in significant elevation of systolic blood pressure only in male rats [46], and that low-level prenatal lead exposure in mice resulted in several permanent male-specific motor deficits [47]. The mechanisms underlying the susceptibility to lead in males are still unclear. The ratio of male-to-female births has been declining in several industrial countries since the 1950s [56-58]. In Japan, the male/female ratio of fetal deaths has increased since the 1970s, reaching over 2.0 in 1996 [59]. This trend suggests prenatal vulnerability of the male fetus to environmental changes, particularly at early stages of gestation [60]. A recent review by Clifton [61] suggested that sex differences in growth and survival of the fetus are mediated by

the sex-specific function of the human placenta. In addition, the minimum BPb appeared to be higher in males than females in the present study (Figure 1), although there were no significant difference in BPb between the two sexes. Although the reason for this potential difference is unclear, it may have contributed to the results observed in the present study. The reason for female-dominant effects of lead in animals observed in several studies [48, 49] remains to be elucidated.

In the present study, there was a very low rate of smoking and most participants were of high socioeconomic status (Table 1). Moreover, participants in the present study were healthy pregnant women who gave birth to healthy newborns. Lead therefore seems to be the only risk factor for lowering birth weight in the present study. In addition, birth weight is influenced by a variety of environmental factors, such as smoking [62-64], drinking [62], low socioeconomic status [65], low maternal BMI [66], and low pregnancy weight gain [67]. Although tobacco smoking during pregnancy increases maternal BPb [64], only five mothers were smokers in the present study; the source(s) of lead exposure among the mothers appeared to be passive smoking, drinking water or diet. In addition, U-shaped changes in BPb during pregnancy were observed as has previously been reported [38-40], although this effect was very small and not statistically significant probably due to the very low levels of maternal BPb in the present study. The reason for, and biological significance of, such a change in BPb remains to be investigated. To confirm the reproductive effects of very-low level lead exposure, including the observations reported in the present study, studies under the internationally standardized quality control of BPb measurement are essential.

In summary, the present study suggests that very low-level lead exposure at an early stage of gestation is a risk factor for reduced birth weight. It seems inappropriate to estimate a "safe" concentration of blood lead in pregnancy; women who are of reproductive age should avoid lead exposure, even at what are currently considered acceptable levels. Because birth

weight is a multi-factorial problem, further epidemiological and/or clinical studies may be required to confirm the findings of the present study.

Conflict of interest statement

The authors declare that there are no conflicts of interest in this research.

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Figure 1. Correlation between maternal blood lead concentration (BPb) at 12 weeks' gestation and birth weight in 197 male and 189 female newborns. rs = Spearman's rank correlation coefficient.

Figure 2. Relationship of birth weight to maternal blood level (BPb) at 12 weeks' gestation in 197 male and 189 female newborns, adjusted for possible confounding variables listed in Table 2 by using unstandardized partial regression coefficients:
Birth weight = - 2773.84–253.61 logBPb (12 weeks' gestation) + 10.87 hematocritt (12 weeks' gestation) + 6.73 Maternal age + 17.94 maternal BMI + 123.79 gestational age - 62.88 drinking (males), and = - 3179.33–157.14 logBPb (12 weeks' gestation) + 0.40 hematocritt (12 weeks' gestation) + 9.076 maternal age + 19.06 maternal BMI + 137.91 gestational age + 201.42 drinking (females). Spearman's rank correlation coefficient (rs) between logBPb and adjusted birth weight is shown.

Figure 1.

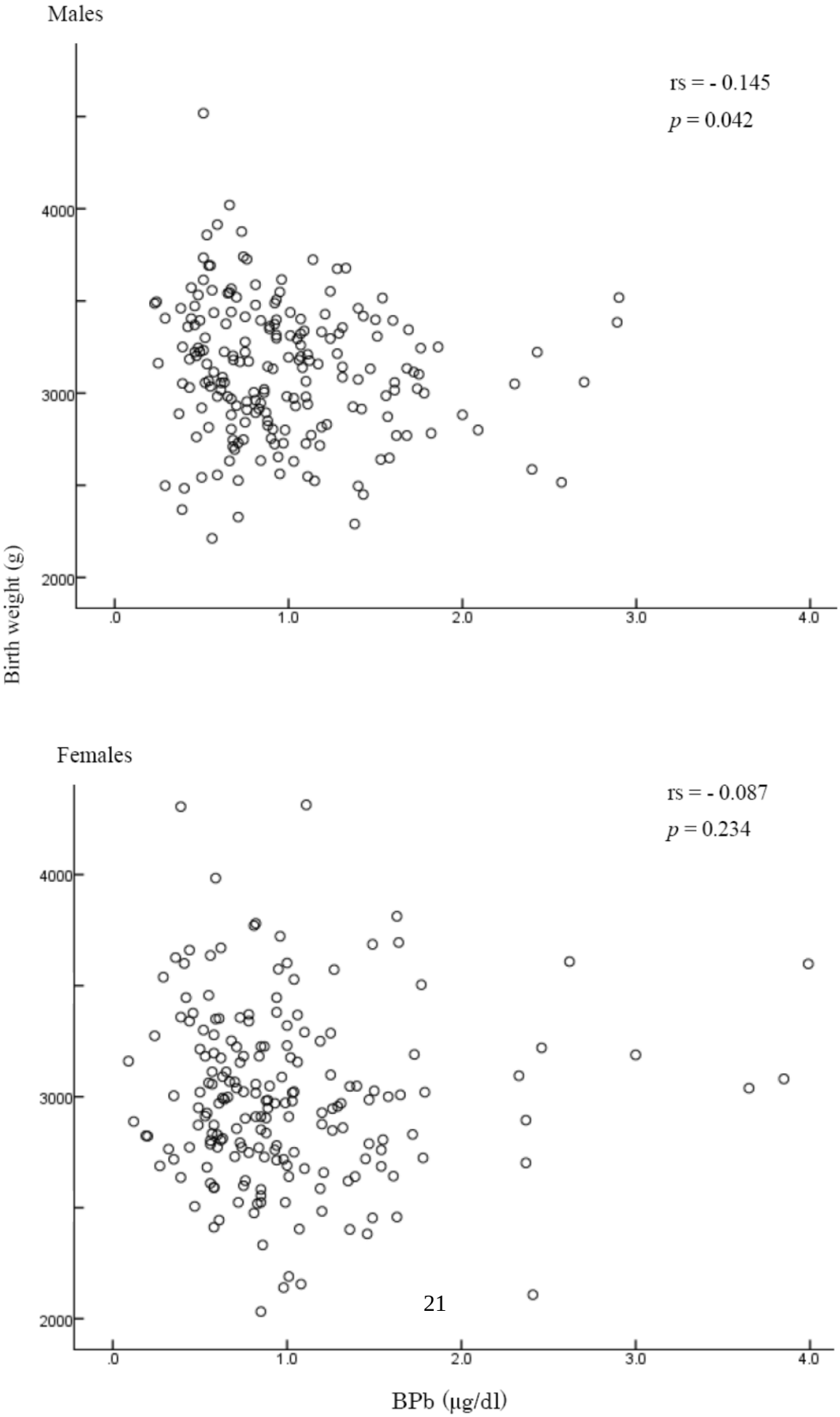
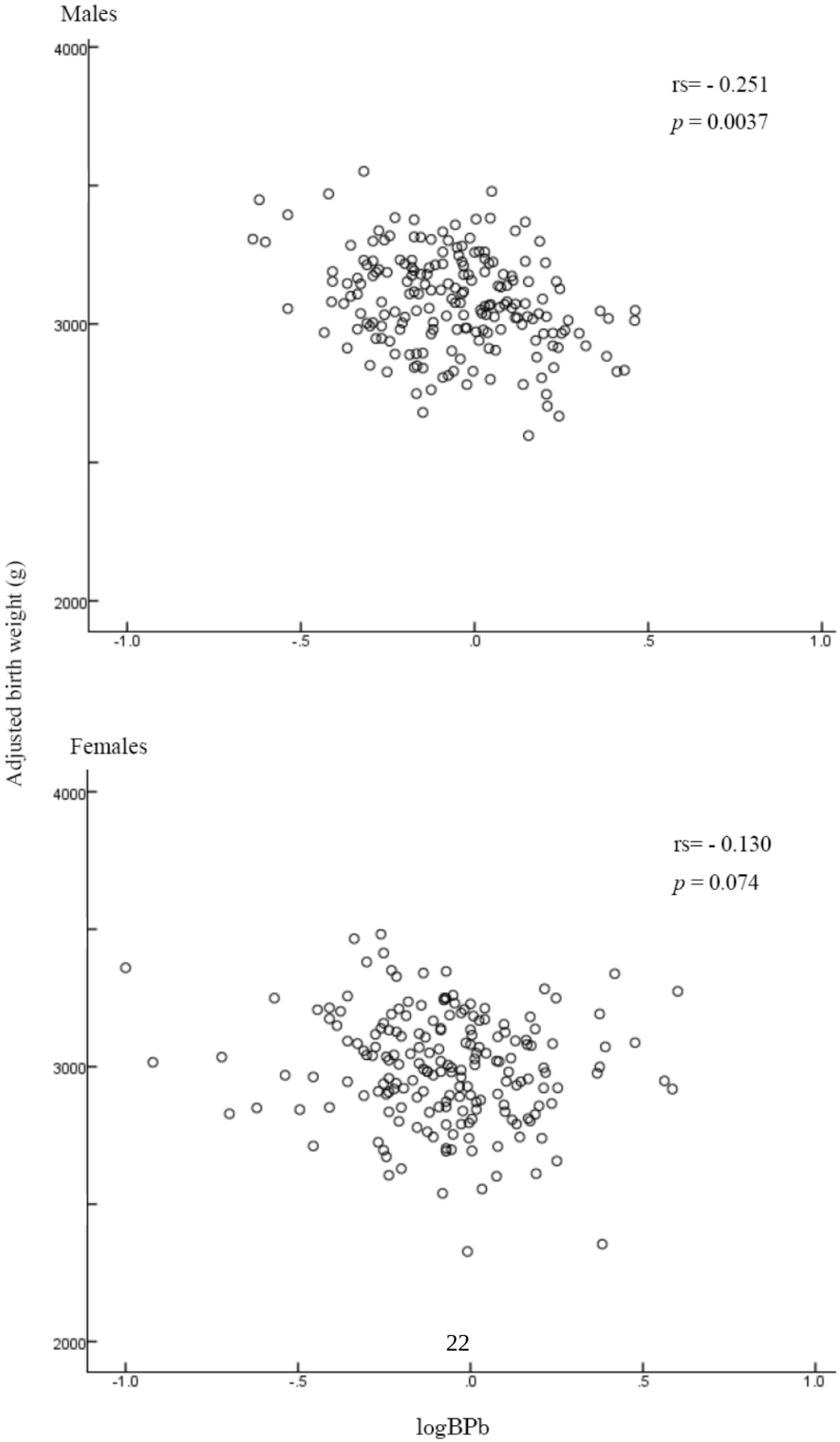

Males
rs = - 0.145
p = 0.042
Birth weight (g)
4000
3000
2000
.0
1.0
2.0
3.0
4.0
Females
rs = - 0.087
p = 0.234
4000
3000
2000
.0
1.0
2.0
3.0
4.0
BPb (µg/dl)

Figure 2.


Males
rs= - 0.251
p = 0.0037
Females
rs= - 0.130
p = 0.074
Adjusted birth weight (g)
4000
3000
2000
-1.0
-.5
.0
.5
1.0
logBPb

548 **Table 1.** Characteristics of 386 mothers included in the final analysis (mean with standard
549 deviation and range in parentheses, or number with the percentage in parentheses).

Age[a]	34.5	(4.8, 21-46)
Height (cm)[a]	159.3	(5.1, 141-175)
Body weight (kg)[a]	62.4	(7.5, 35-85)
Body mass index (kg/m^2)[a]	24.6	(2.7, 18.8-39)
Blood lead (μg/dl)		
12 weeks	0.98	(0.55, 0.10-3.99)
25 weeks	0.92	(0.63, <0.09-3.96)
36 weeks	0.99	(0.66, <0.09-3.96)
Hematocrit (%)		
12 weeks	36.3	(2.7, 28.8-43.1)
25 weeks	33.4	(2.3, 24.4-39.2)
36 weeks	33.8	(4.2, 26.0-40.9)
Vaginal delivery	281	(72.8)
Smoking during pregnancy	5	(1.3)
Alcohol consumption before pregnancy	274	(71.4)
Alcohol consumption during pregnancy[b]	11	(2.8)
Education levels		
High school	28	(7.4)
College	120	(31.7)
University or more	231	(60.9)
Annual household income (≥ 5million yen)	316	(85.6)

550 [a] Immediately prior to delivery
551 [b]At 12 weeks' of gestation
552

Table 2. Relationships of birth weight to maternal blood lead level (BPb) at 12, 25 and 36 weeks' gestation, and to other variables, in 197 male and 189 female newborns (standardized partial regression coefficient)[a]: multiple regression analysis.

	logBPb[a]	Hematocrit[a]	Age[b]	Body mass index[b]	Gestational age[b]	Drinking[c]	Adjusted R^2
Males:							
12 weeks	- 0.151*	0.079	0.087	0.118	0.426***	- 0.024	0.223***
25 weeks	- 0.003	-0.078	0.066	0.157*	0.403***	- 0.016	0.207***
36 weeks	0.051	-0.057	0.064	0.144*	0.402***	- 0.011	0.206***
Females:							
12 weeks	- 0.098	0.003	0.116	0.146*	0.474***	0.098	0.265***
25 weeks	- 0.031	-0.154*	0.071	0.163*	0.478***	- 0.081	0.280***
36 weeks	- 0.054	-0.046	0.094	0.159**	0.469***	- 0.096	0.261***

[a]12, 25 and 36 weeks' gestation, respectively.

[b] Immediately prior to delivery.

[c] At 12 weeks' gestation. (Yes=1, No=0).

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$