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Title Page

First trimester serum folate levels and subsequent risk of abortion and preterm birth among Japanese women with singleton pregnancies

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running title

Serum folate levels and preterm birth among Japanese women

Abstract

Objectives: To determine whether a low serum folate level during the first trimester predicts subsequent late abortion, preterm birth, or fetal growth restriction (FGR).

Study design: A prospective cohort study involving 5,075 women whose serum folate levels were measured during the first trimester. The participants were informed of their serum folate levels.

Results: The pregnancy duration, birthweight, rate of late abortion/preterm birth, and the rate of FGR did not differ significantly among four groups classified according to folate status. The mean serum folate levels did not differ among quartiles classified according to the gestational week at the time of delivery. Nineteen of the 20 women with folate deficiency gave birth at term to infants with a birthweight of 3,132 ± 321 g; only one infant had FGR.

Conclusion: Low serum folate levels during the first trimester were not associated with the risk of late abortion, preterm birth**,** or FGR.

Key words

abortion, nutrition, preterm birth, serum folate

Introduction

Adequate folate status is important not only during the periconceptional period, but also throughout pregnancy to achieve a satisfactory pregnancy outcome [1]. Since unambiguous evidence has shown that folic acid protects against both the first occurrence and the recurrence of neural tube defects [2,3], expert committees worldwide have issued folic acid recommendations. In 2000, the Japanese government also issued a recommendation that all women planning to conceive consume a daily supplement containing 400 μg of folic acid to reduce the risk of neural tube defects during pregnancy.

Because folate is essential for DNA and RNA biosynthesis, an increased demand for folate exists during pregnancy because of the cellular proliferation occurring in the placenta and fetus. Indeed, the amount of folate breakdown products in the urine doubles during the second trimester and returns to the baseline level postpartum, suggesting an extra demand for dietary folate during pregnancy [4]. Since the calculated total fetal and placental folate content is 800 μg at term [5], the increased demand for folate may be due not only to fetal transfer, but also to the accelerated breakdown of the vitamin through folate's participation in DNA synthesis [6]. This hypothesis suggests a possible relation between the folate status in the mother and fetal growth. Indeed, the effects of folate on increases in birthweight have been observed with some consistency among poorly nourished women [7, 8].

Folate is also required for homocysteine metabolism. Folate is the major determinant of homocysteine concentrations, with well-established evidence that supplementation with folic acid has a marked homocysteine-lowering effect [9]. Folic acid supplementation during pregnancy decreases the plasma total homocysteine level [10].

Thus, the serum homocysteine level can be used as a marker of folate status. Hyperhomocysteinemia is associated with pregnancy complications and an adverse pregnancy outcome, including miscarriage [11], placental abruption [12], and severe preeclampsia [13]. Since all these complications may shorten the length of gestation, the preterm birthrate may be higher among women with folate deficiency than among those with a normal folate status. Indeed, women with serum folate levels < 16.3 ng/mL (<37.3 nmol/L) during the second trimester have a higher risk of preterm birth than women with folate levels ≥ 16.3 ng/mL in results of a previous study [14] in which, however, no information regarding frequency of peeclampsia or placental abruption is provided. Whether the folate status during the first trimester is associated with the risk of preterm birth or inadequate fetal growth remains unknown. Accordingly, we conducted this prospective cohort study to examine whether the first trimester serum folate level is associated with preterm birth or fetal growth.

Methods

This study was performed after receiving the approval of our institutional review board and obtaining informed consent from all the participants. The study participants were native Japanese pregnant women recruited from an ongoing birth cohort: The Hokkaido Study on Environment and Children's Health. The cohort was started in February 2003 and recruitment is ongoing; the details of the study have been described previously elsewhere [15]. All pregnant indigenous Japanese women who were booked to receive antenatal care at any of 37 participating health facilities in Hokkaido were considered eligible for the cohort study. This report analyzed data from 5,075 participants who were recruited during their first trimester between February 2003 and

March 2006. Data on the demographic characteristics were obtained from a baseline self-administered questionnaires at the time of recruitment and the infant's hospital birth records. We were able to collect information including maternal age, number of prior births, body height, pre-pregnancy body weight, folate supplementation, smoking alcohol ingestion, annual income, gestational week at delivery and infant birth-weight. Small for gestational age (SGA) was defined as a birthweight below the 10^{th} percentile for gestational age for Japanese infants [16].

Serum folate concentrations were assayed in the blood specimens obtained during the first trimester (gestational week [GW] of $5^{-0/7}$ to $13^{-6/7}$) using an automated competitive protein binding chemiluminescent enzyme immunoassay (CLEIA) technique according to the assay manufacturer's instructions; the measurements were performed by a commercial laboratory (SRL, Inc. Tokyo, Japan). Analyses were conducted in batches because of the continuous recruitment of the study participants. The laboratory personnel were blinded to the folate supplement status of the participants whose biological specimens were assayed. Each participant's physician informed the participant of her serum folate level approximately three weeks after the blood sample collection.

Statistical analyses were performed using the JMP8 statistical software package (SAS, Cary, NC). Data are reported as mean ± SD, unless otherwise stated. The Mann-Whitney U and the Kruskal-Wallis tests were used when comparing two or three groups, respectively. Differences in the means were tested using the Tukey-Kramer HSD (honestly significant difference) test between each group, and categorical variables were compared using Pearson's chi-squared test. A P value of less than 0.05 was considered to indicate statistical significance.

Results

Among the 5,075 women analyzed in the present study, the mean serum folate level determined at GW 10.7 ± 1.5 was 18.4 ± 21.6 nmol/L (Table 1). Forty-eight (1.0%) and 220 (4.3%) women experienced a late abortion between GW $14^{-0/7}$ and $21^{-6/7}$ and preterm birth between GW $22^{-0/7}$ and $36^{-6/7}$, respectively. The intake of folate supplements significantly increased the serum folate levels (Table 1) and the number of women with higher serum folate levels (Fig. 1). Serum folate levels (nmol/L) were 16.9 ± 13.8 in 4499 women with no folate supplementation, 29.2 ± 59.2 in 389 women who began to take folate supplementation after conception, and 36.0 ± 19.0 in 129 women who initiated folate supplementation before conception (Table 1). Serum folate levels increased with advancing maternal age (Table 1 and Fig. 2). In addition, the pre-pregnancy maternal body mass index, use of infertility treatment, alcohol ingestion, and annual income were associated with the serum folate levels. Women with a smoking habit had a significantly lower serum folate level than non-smokers. Multiple linear regression analysis was performed with serum folate concentration as the dependent variable and maternal age, prepregnancy BMI, infertility treatment, folate supplementation, smoking, alcohol intake, and annual income as independent variables, resulting that only folate supplementation was statistically significant variable ($p=0.0004$). None of the maternal characteristics were significantly associated with the rate of late abortion or preterm birth (Table 1).

The effect of folate status during the first trimester on the subsequent preterm birth and fetal development was examined. The rate of late abortion/preterm birth, the mean gestational week at delivery, the mean birthweight, the SGA rate, or the

female-to-male ratio did not differ significantly among quartiles divided according to folate status (Table 2). Unexpectedly, the mean serum folate level was significantly higher among women who gave birth at a GW of 28 to 36 than among women who gave birth at term (Table 3). The outcomes of the 20 women with folate deficiency (< 6.8 nmol/L) were investigated individually. One woman miscarried at GW 20, but the remaining 19 women gave birth to infants with a birthweight of 3,132 ± 321 g (mean ± SD) at term (GW 39.5 ± 1.1). Only one of them gave birth to an SGA infant weighing 2550 g at GW 38.

Comment

Unexpectedly, the present study indicated that the serum folate level during the first trimester was not associated with either preterm birth, including late abortion, or fetal growth.

As to the possible relationship between serum folate levels and preterm delivery, a series of previous reports have suggested that a low serum folate level during pregnancy increases the risk of premature delivery [14,17,18]. The total homocysteine (tHcy) level measured in serum can be a marker of folate status, suggesting an inverse relationship between serum folate and total homocysteine levels [19]. Methylenetetrahydrofolate reductase (*MTHFR*) is involved in the metabolism of folate and homocysteine: a polymorphism (677C→T) in the gene of *MTHFR* is associated with lower serum folate levels and higher tHcy levels [20, 21]. In the Hordaland Homocysteine Study using a cohort of 5,883 women, a higher plasma tHcy concentration, measured among women aged 40 to 42 years old, was associated with significantly higher incidences of previous preeclampsia and previous premature delivery [17, 18]. In addition, in a prospective

study of 2,026 women with a mean (SD) serum folate level of 21.1 ± 11.0 ng/mL (median, 20.1 ng/mL; 5^{th} – 95^{th} percentile, 6.4 – 41.1 ng/mL) during the second trimester, a significantly larger number of women with serum folate levels < 16.3 ng/mL subsequently gave birth at preterm, compared with women whose serum folate levels were ≥ 16.3 ng/mL [14]. Although the Hordaland Homocysteine Study [17, 18] did not measure the plasma folate levels, all these reports have suggested the possibility that low plasma folate levels during the first trimester predict the risk of preterm birth.

The preterm premature rupture of membranes (PPROM) explains up to one-third of all preterm births [22]. However, the serum level of homocysteine or folic acid measured in patients with PPROM does not differ from that in matched control women [23, 24], raising questions regarding the association between the folate status and preterm birth. In the present study, the participants were not blinded to their folate levels determined during the first trimester: the folate levels were sent to the participants' physicians approximately three weeks after blood collection, and no rules were defined as to whether the physicians should inform the participants of their folate levels or how to explain the folate level results. Some participants may have begun to take folate supplements after being told their serum folate level or after the blood collection independently of their folate status, although information regarding this issue was not available in the present study. If we assume that the women who had a low serum folate status during certain periods of their pregnancies would have a higher risk of preterm birth, such behavioral changes among the participants may have modified the present results, although the effect of folate fortification on the risk of preterm birth has not been demonstrated to date.

As to the possible relationship between the serum folate levels and fetal growth,

many studies have suggested an association between these factors [18, 25-28]. The folate levels in cord blood were significantly lower in infants with FGR than in normal birthweight infants [25]. The percentage of FGR decreases as the serum folate concentration at 30 weeks of gestation increases, in addition to significantly larger birthweight infants born to mothers with a serum folate level above the median value, compared with their counterparts [26]. The risk of FGR increases with the number of T alleles in the (677C→T) polymorphism of the *MTHFR* gene [27, 28] in addition to an increased risk of FGR among women with higher serum tHcy levels in the Hordaland Homocysteine Study [29]. Conflicting results have also been obtained in other studies [29-32]. An elevated tHcy level did not increase the risk of FGR [29, 30]. A maternal 677TT genotype for *MTHFR* was not associated with the risk of FGR [31, 32], but a maternal 1298CC genotype for *MTHFR* reduced the risk of FGR (odds ratio, 0.49; 95% confidence interval, 0.25 – 0.93) [32]. A recent study suggested that a maternal *MTHFR* 1298AA genotype was associated with a low folate status, and only smokers with this genotype had a significantly reduced infant birthweight; meanwhile, carrying the T allele of the *MTHFR* (677C→T) genotype was significantly associated with a low serum folate level, but not with the offspring's birthweight [33], suggesting that smoking and the *MTHFR* (1298A→C) polymorphism have a greater impact on fetal growth than the *MTHFR* (677C→T) polymorphism. These findings may explain why conflicting results have been obtained regarding the relation between the maternal serum folate/tHcy status and fetal growth.

The prevalence of preterm births at < 37 weeks of gestation was relatively low (4.3%) in the present study, with a prevalence of 7.3% reported in the Hordaland Homocysteine Study [18] and 14.2% reported in a study performed in the USA [14]. During the study

period, the number of preterm singleton infants born at or after 22 weeks of gestation was approximately 55,000 to 57,000 annually in Japan, accounting for approximately 5.0% of all newborn infants (Tabulated information released annually by the Japanese Ministry of Health, Labour and Welfare). Thus, although Japan has a relatively low incidence rate of preterm births compared with other countries, the reason why the preterm birthrate is low in Japan remains unknown. Approximately 0.3% of pregnant women do not receive adequate antenatal care in Hokkaido, and such women with no adequate antenatal care have an increased risk of preterm birth [34]. In the present study, the study subjects were pregnant women who received regular antenatal care from an early stage of pregnancy. This situation may have provided a favorable bias, resulting in a smaller number of women with preterm births in the present study compared with the general statistics for Japan (4.3% versus approximately 5.0%).

In conclusion, the prospective cohort study involving 5,075 pregnant women with known first-trimester folate levels demonstrated that neither the pregnancy duration, birthweight, rate of late abortion/preterm birth, nor the rate of FGR differed significantly among four groups classified according to the first-trimester folate status. In addition, the mean serum folate levels did not differ among quartiles classified according to the gestational week at the time of delivery. These results suggested that low serum folate levels during the first trimester were not associated with the risk of late abortion, preterm birth, or FGR. However, the present study did not preclude a possibility that active folate intake at and after 2nd trimester in women with lower folate levels modified the pregnancy duration and or fetal growth.

Conflict of Interest

We declare that we have no conflict of interest.

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Figure Legends

Figure 1: Frequency of serum folate levels among women with or without folate supplementation.

Figure 2: Relationship between maternal age and serum folate levels.

Fig. 1

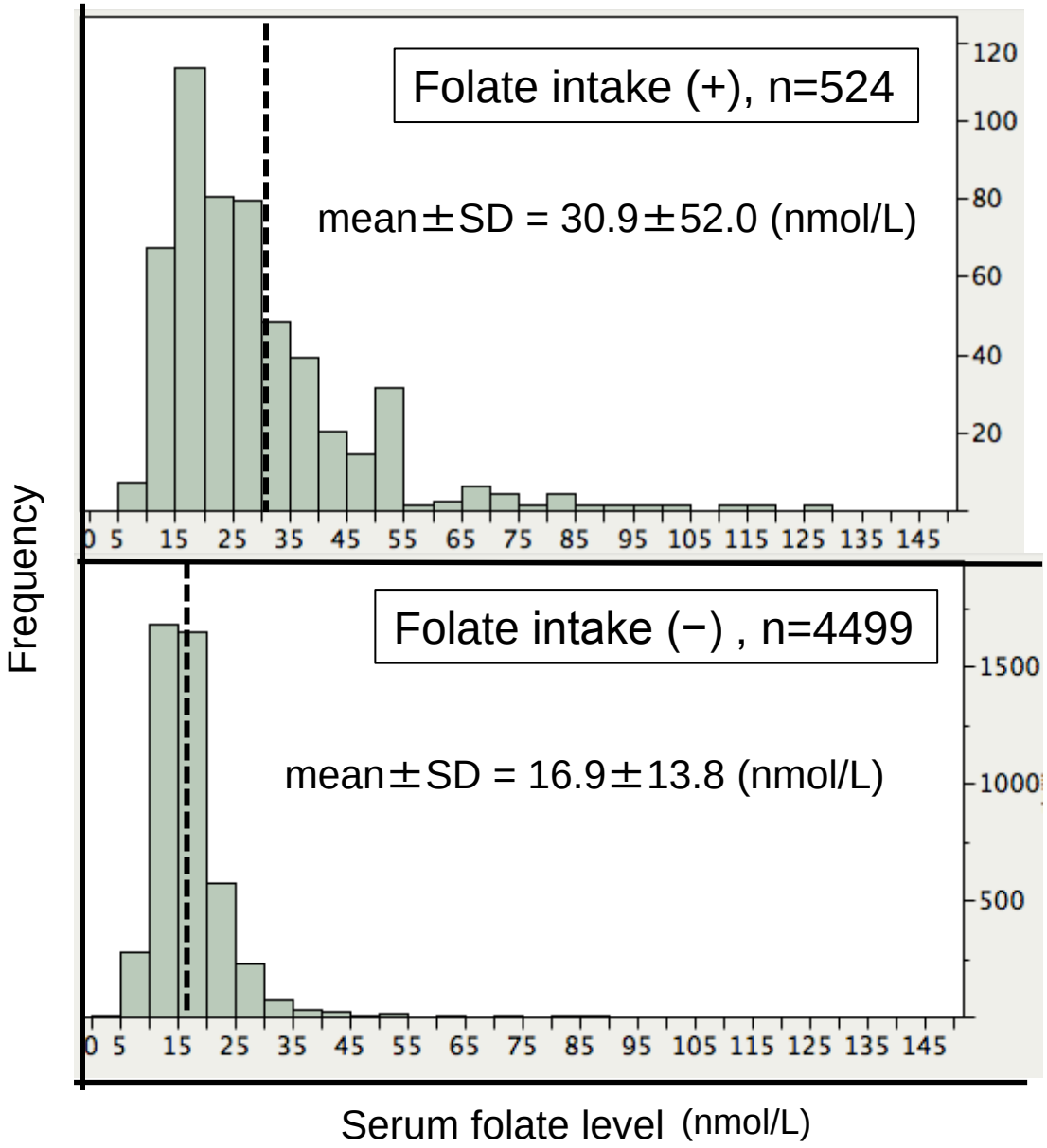

Folate intake (+), n=524
mean±SD = 30.9±52.0 (nmol/L)
120
100
80
60
40
20
0 5 15 25 35 45 55 65 75 85 95 105 115 125 135 145
Folate intake (−) , n=4499
mean±SD = 16.9±13.8 (nmol/L)
1500
1000
500
0 5 15 25 35 45 55 65 75 85 95 105 115 125 135 145
Frequency
Serum folate level (nmol/L)

Fig. 2 , （n=4499）

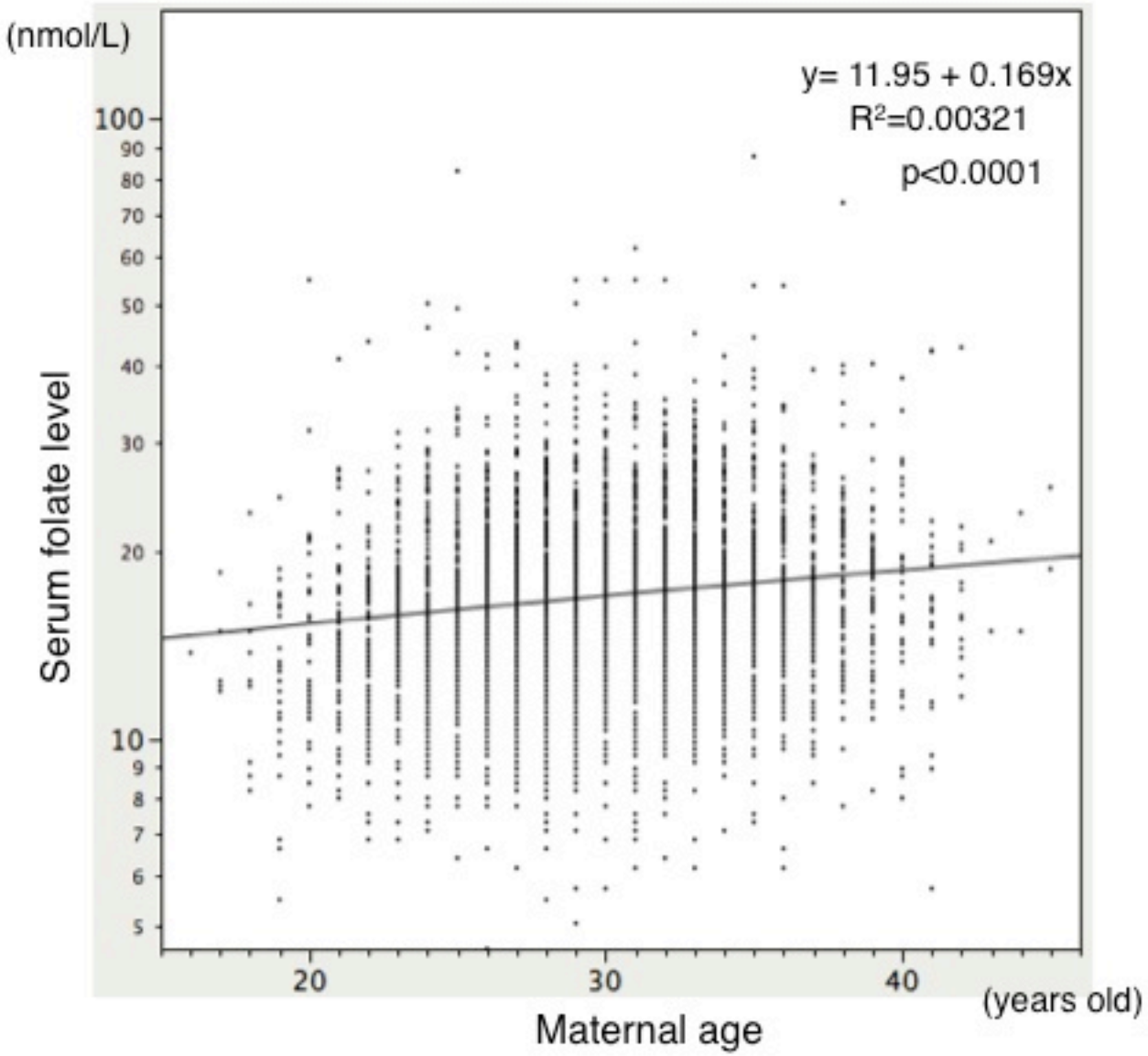

(nmol/L)
y= 11.95 + 0.169x
R²=0.00321
p<0.0001
100
90
80
70
60
50
40
30
20
10
9
8
7
6
5
Serum folate level
20
30
40
(years old)
Maternal age

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

		Serum folate level (nmol/L)			p-value*	Late abortion	p-value (χ2)	Preterm delivery	p-value (χ2)
Overall	(n=5075)	18.4	±	21.6		48 (1.0%)		220(4.3%)	
Maternal age (years)					<0.0001		0.0825		0.3626
≤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%)	
prepregnancy BMI (kg/m2)					0.0019		0.6611		0.1617
<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%)	
Parity					0.4237		0.1587		0.8739
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%)	
Infertility treatment					0.0006		0.3367		0.4556
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%)	
Folate supplementation					<0.0001		0.9888		0.4694
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%)	
Smoking					<0.0001		0.3691		0.6460
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%)	
Alcohol ingestion					0.0060		0.9512		0.0971
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%)	
Annual income (USD)					<0.0001		0.0817		0.7985
<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%)	

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

BMI: Body mass index

†: p=0.0005 vs. age 30-39 years, ‡: p= 0.0089 vs. no folate supplementaion, ¶ : p<0.0001 vs. no folate supplementaion and folate supplementaion after conception

*: The Mann-Whitney U and the Kruskal-Wallis tests were used when comparing the means in two or three groups, respectively.

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		p-value†	Gestational age at delivery (week)			p-value‡	Birthweight (g)			p-value‡	SGA (<10%tile)	p-value†	girl/boy	p-value†
Overall	(n=5075)	268	(5.3%)		38.5	±	2.9		3020	±	485		7.1%		1.012	
≤13.1	(n=1244)	71	(5.7%)	0.6928	38.5	±	3.0	0.2531	3007	±	474	0.2372	5.5%	0.1135	1.052	0.3968
13.2-16.1	(n=1303)	72	(5.5%)		38.5	±	3.1		3008	±	528		7.6%		0.940	
16.2-20.0	(n=1273)	60	(4.7%)		38.7	±	2.4		3042	±	447		6.8%		1.003	
≥20.1	(n=1255)	65	(5.2%)		38.5	±	2.8		3021	±	487		8.4%		1.065	

†: χ2-test, ‡: ANOVA

SGA: small for gestational age

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

Gestational 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