Validity and reproducibility of folate and vitamin B₁₂ intakes estimated from a self-administered diet history questionnaire in Japanese pregnant women

Mie Shiraishi¹*, Megumi Haruna¹, Masayo Matsuzaki¹, Ryoko Murayama¹, Satoshi Sasaki² and Sachiyo Murashima³

Abstract

Background: No validated dietary questionnaire for assessing folate and vitamin B₁₂ intakes during pregnancy is available in Japan. We evaluated the validity and reproducibility of intakes of folate and vitamin B₁₂ estimated from a self-administered diet history questionnaire (DHQ) in Japanese pregnant women.

Methods: A sample of 167 healthy subjects with singleton pregnancies in the second trimester was recruited at a private obstetric hospital in metropolitan Tokyo from June to October 2008 (n = 76), and at a university hospital in Tokyo from June 2010 to June 2011 (n = 91). The dietary intakes of folate and vitamin B₁₂ were assessed using the DHQ. The serum concentrations of folate and vitamin B₁₂ were measured as reference values in the validation study. To assess the reproducibility of the results, 58 pregnant women completed the DHQ twice within 4-5 week interval.

Results: Significantly positive correlations were found between energy-adjusted intakes and serum concentrations of folate and vitamin B₁₂ (r = 0.286, \( p < 0.001 \) and r = 0.222, \( p = 0.004 \), respectively). After excluding the participants with nausea (n = 121), the correlation coefficient for vitamin B₁₂ increased to 0.313 (\( p = 0.001 \)). When participants were classified into quintiles based on intakes and serum concentrations of folate and vitamin B₁₂, approximately 60% were classified in the same or adjacent quintile. The intraclass correlation coefficients of the two-time DHQ were 0.725 for folate and 0.512 for vitamin B₁₂.

Conclusion: The present study indicated that the DHQ had acceptable validity and reproducibility for assessing folate and vitamin B₁₂ intakes in Japanese pregnant women.

Keywords: Diet history questionnaire, Pregnant women, Validation, Folate, Vitamin B₁₂

Background

Low birth weight is a major cause of perinatal morbidity and low birth weight babies are susceptible to cardiovascular disease in later life [1]. The incidence of low birth weight increased in Japan from 6.3% in 1990 to 9.6% in 2007 [2]. One possible reason for the low birth weight is low intake of micronutrients during pregnancy [3,4]. For instance, folate and vitamin B₁₂ deficiency can lead to retardation of fetal development through hyperhomocysteinemia, which negatively affects DNA methylation and cell proliferation [5-8]. Assessment of an individual’s nutritional status including folate and vitamin B₁₂ is therefore important to prevent the harmful effects of improper nutrient intake on pregnancy outcome.

The methods used for assessment of dietary intake often include weighed food records, measurement of certain blood markers, and 24-hour urine analysis [9]. However, these methods may be costly and can impose a significant burden on participants. Therefore, easier and less onerous methods are needed for screening and large-scale research studies, such as the dietary

* Correspondence: mi-shi@umin.ac.jp

¹Department of Midwifery and Women’s Health, Division of Health Sciences and Nursing, Graduate School of Medicine, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

Full list of author information is available at the end of the article

© 2012 Shiraishi et al; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
assessment questionnaire. This convenient method is used commonly in epidemiological studies to identify individuals with low and high nutrient intake [9]. However, no validated dietary assessment questionnaire exists in Japan for pregnant women, making it difficult to implement large interventional studies and epidemiologic studies regarding prenatal nutrition.

A self-administered diet history questionnaire (DHQ) developed in Japan to evaluate nutrient intakes in both healthy individuals and high-risk populations has been validated only in non-pregnant populations [10,11]. A separate validation study of the DHQ for pregnant women is therefore required, particularly given that dietary habits are likely to change in both pregnant women and their babies, and dietary intake is affected by physical conditions associated with pregnancy, and changes in appetite and feeling [12].

The present study was designed to assess the relative validity and reproducibility of the DHQ among Japanese pregnant women, using serum biomarkers of folate and vitamin B12. The study also compared folate and vitamin B12 intakes estimated from two-time DHQ.

Methods
Overview of recruitment and study design
The present study was conducted at a private obstetric hospital (S hospital) in metropolitan Tokyo, Japan, between June and October 2008, and at a university hospital (T hospital) in Tokyo between June 2010 and June 2011. Neither hospital routinely conducts individualized guidance of nutrient intake for low-risk pregnant women. For this study, healthy Japanese women with singleton pregnancies were surveyed during the second trimester. The exclusion criteria included patients with hyperemesis and hypertension to avoid their burden of participation in the study in consideration of their symptoms. We also excluded women with diabetes and those enrolled in formal nutrition training because they had done other interviews regarding their intakes and had received nutritional intervention. Other exclusion criteria included age younger than 20 years, poor ability reading Japanese, and intake of supplements including folic acid and vitamin B12. All women underwent ultrasonography at 8-12 weeks gestation to allow accurate dating of gestation. All participants were provided with detailed information about the study protocol, and each gave a written informed consent. The Ethics Committee of the Graduate School of Medicine at the University of Tokyo approved the study procedures and protocol.

Each participant completed the questionnaire while waiting for a pregnancy checkup (S hospital: 24-27 weeks gestation, T hospital: 19-23 weeks gestation). Participants who did not have sufficient time to complete the questionnaires filled them out after returning home, and submitted them by mail. We resolved missing and unclear data directly when questionnaires were submitted to researchers or by telephone interview, and reviewed participants’ medical charts to obtain information on the pregnancy. To reduce subjects’ burden, we drew non-fasting blood samples at the hospitals during collection of biological samples for routine blood tests that were conducted in association with a normal pregnancy health examination (S hospital: 24-27 weeks gestation; T hospital: 19-23 weeks gestation). The participants answered questionnaires including the DHQ on the day of blood sample collection or within 7 days after the blood sample collection.

To assess the reproducibility of the DHQ, pregnant women at 15-19 weeks gestation were recruited at T hospital between February and April, 2011 and asked to complete the DHQ twice in the second trimester; the first DHQ was completed at recruitment and the second DHQ 4-5 weeks later.

Diet history questionnaire
The DHQ was designed to assess dietary intake over the previous month using a questionnaire developed and validated for the Japanese adult population [10,11]. The DHQ is a 22-page structured questionnaire that measures the daily intake of 150 foods, and calculates the intakes of energy, folate, and vitamin B12 using an ad hoc computer algorithm based on the Japanese standard food composition tables [13]. Items and portion sizes were derived from primary data of the National Nutrition Survey of Japan and from various Japanese recipe books for Japanese dishes [10]. The daily intakes of vitamins were calculated as nutritional amounts in the foods consumed multiplied by the frequency and portion size. Eight frequency responses were listed ranging from more than twice per day to almost never. Five portion size responses were listed from less than half to more than 1.5 times a general portion size. These standard values were established as part of the initial development of the DHQ for non-pregnant populations.

We excluded participants who reported an extremely unrealistic energy intake; that is, the reported energy intake was less than half the energy requirement for the lowest physical activity category or more than 1.5 times the energy requirement for moderate physical activity according to the “Dietary Reference Intakes for Japanese” [14,15].

Biological markers
Blood samples were centrifuged for 10 minutes at 3000 rpm to separate serum within 6 hours after blood collection, and the serum samples were stored at -80°C until analysis. The samples were measured within 3
months after collection. Serum concentrations of folate and vitamin B₁₂ were assayed by chemiluminescent immunoassay using ADVIA Centaur® (Siemens AG, Munich, Germany) with Chemilumi-ACS folate II and Chemilumi-ACS vitamin B₁₂ kits (Siemens Healthcare Diagnostics Co. Ltd., Tokyo, Japan). These assays were conducted by Mitsubishi Kagaku Bio-Chemical Laboratories, Tokyo, Japan.

**General questionnaires and data processing**

We collected demographic and lifestyle information from the study participants, such as age, gestational age and educational levels, using a self-administered questionnaire. Education level was categorized as < 13, 13-15, or > 15 completed schooling years. Prepregnancy body mass index (BMI) was calculated from self-reported prepregnancy weight and height. The participants were classified as underweight (BMI < 18.5 kg/m²), normal weight (18.5 ≤ BMI < 25.0 kg/m²) and overweight or obese (BMI ≥ 25.0 kg/m²) based on the criteria of the World Health Organization. The pregnant women were also asked about nausea and vomiting during pregnancy.

**Statistical analysis**

**Validation study**

We assumed that the level of validity between intakes estimated from the DHQ and their corresponding serum concentrations was more than $r = 0.40$, with 80% power and a 5% significance level. A sample size of more than 123 would be required to detect a minimally acceptable level of $r = 0.25$ [16]. We compared parameters between the two hospitals using Student’s t-test or the chi-square test. The Shapiro-Wilk test was used to test for normal distribution of the continuous variables. We used the density method for energy-adjustment of vitamins intake. Partial correlation analyses were conducted to test for associations between dietary intakes and serum concentrations of folate and vitamin B₁₂, adjusting for research settings. These analyses were conducted after all variables were log-transformed to achieve normal distributions.

All pregnant women were classified into quintiles according to their intakes and serum concentrations for each vitamin. Agreement and discordance in quintile ranking was assessed as the percentage classification for pregnant women based on the quintiles according to folate and vitamin B₁₂ intakes estimated from the two-time DHQ. Agreement and discordance in quintile ranking was assessed as the percentage classification for pregnant women based on the two-time DHQ in the same, adjacent, and opposite quintiles. We also calculated mean vitamins intakes estimated from the second DHQ in each quintile based on the first DHQ. Linear regression analyses were performed to test for significant linear trends between estimated intakes from the two-time DHQ.

Bland-Altman plots were used to illustrate the difference between the two-time DHQ against the mean intakes by the two-time DHQ [17]. The upper and lower lines represented the upper and lower 95% limits of agreement (mean difference ± 1.96 SD). All statistical analyses were conducted using The Statistical Package for Social Sciences for Windows, version 15.0 (SPSS Japan Inc.). All statistical tests were two-sided and $P$ values less than 0.05 were considered statistically significant.

**Results**

**Validation study**

We recruited 358 pregnant women (S hospital: 128, T hospital: 230) and 322 (89.9%; S hospital: 114, T hospital: 208) of these gave their written informed consent. We excluded 128 pregnant women who were taking folic acid or vitamin B₁₂ supplements (S hospital: 27, T hospital: 101) and further 27 women for the following reasons: 12 had missing data; 8 had a severe under-reported energy intake; and 7 had outlier data such as high serum concentrations despite low intakes. Our final analysis was therefore of data from 167 healthy singleton pregnant women (46.6%; S hospital: 76, T hospital: 91).

Table 1 summarizes the characteristics of the participants. Participants in the T hospital had a higher mean age than those in the S hospital, and there were significant differences in education level and household income between the two hospitals.
Table 2 summarizes the mean vitamins intake estimated from the DHQ and mean serum concentrations. Significant differences were found in daily folate intakes, energy-adjusted folate intakes, serum folate concentrations, and serum vitamin B12 concentrations between the two hospitals.

Both the daily intakes and energy-adjusted intakes of the two vitamins correlated positively with the corresponding serum concentrations (Table 3). When participants with reported nausea were excluded \((n = 121)\), the correlation coefficient between energy-adjusted intake and serum concentration of vitamin B12 increased from

Table 1 Characteristics of participants

|                      | Validation study | Reproducibility study |       |
|----------------------|------------------|-----------------------|-------|
|                      | All participants \((n = 167)\) | S Hospital \((n = 76)\) | T Hospital \((n = 91)\) |
|                      | Mean ± SD or n (%) | Mean ± SD or n (%) | Mean ± SD or n (%) | \(p^1\) | Mean ± SD or n (%) |
| Age [years]          | 32.4 ± 4.9        | 30.5 ± 5.0            | 34.1 ± 4.1        | < 0.001 | 33.8 ± 4.2        |
| Parity: Primigravida \([n \%(\%)]\) | 80 (48)          | 34 (45)               | 46 (51)           | ns      | 38 (66)           |
| Currently married \([n \%(\%)]\) | 166 (99)         | 75 (99)               | 91 (100)          | ns      | 58 (100)          |
| Education \([n \%(\%)]\) | < 13 years       | 32 (19)               | 25 (33)           | < 0.001 | 7 (12)            |
|                      | 13-15 years      | 75 (45)               | 37 (49)           | 24 (41) |
|                      | > 15 years       | 60 (36)               | 14 (18)           | 31 (53) |
| Household income (Japanese yen/year) \([n \%(\%)]\) | < 5,000,000      | 59 (35)               | 38 (50)           | < 0.001 | 7 (12)            |
|                      | 5,000,000-9,000,000 | 70 (42)             | 32 (42)           | 27 (47) |
|                      | > 9,000,000      | 38 (23)               | 6 (8)             | 24 (41) |
| Height [cm]          | 158.7 ± 5.7      | 157.8 ± 5.2           | 159.5 ± 5.9       | 0.048   | 159.1 ± 5.4       |
| Prepregnancy BMI\(^2\) [kg/m\(^2\)] | 20.5 ± 2.3       | 20.6 ± 2.3            | 20.3 ± 2.4        | ns      | 20.2 ± 3.0        |
|                      | BMI < 18.5 \([n \%(\%)]\) | 28 (17)             | 8 (11)            | 20 (22) | ns                | 17 (29) |
|                      | 18.5 ≤ BMI < 25.0 \([n \%(\%)]\) | 131 (78)           | 65 (86)           | 66 (73) | 38 (65)           |
|                      | BMI ≥ 25.0 \([n \%(\%)]\) | 8 (5)               | 3 (4)             | 5 (6)   | 3 (5)             |
| Working \([n \%(\%)]\) | 66 (40)          | 21 (28)               | 45 (50)           | 0.004   | 22 (38)           |
| Regular smoker during pregnancy \([n \%(\%)]\) | 3 (2)            | 1 (1)                 | 2 (2)            | ns      | 1 (2)             |
| Regular alcohol drinker during pregnancy \([n \%(\%)]\) | 5 (3)            | 2 (3)                 | 3 (3)            | ns      | 2 (3)             |
| Nausea during the study period \([n \%(\%)]\) | 46 (28)          | 15 (20)               | 31 (34)          | 0.039   | 10 (17)           |
| Gestational age [weeks] | 22.6 ± 2.8       | 25.4 ± 1.0            | 20.3 ± 1.2        | < 0.001 |
| Gestational age at first survey [weeks] | 169 ± 1.5        |                     |                   |         |
| Gestational age at second survey [weeks] | 210 ± 1.3        |                     |                   |         |

\(^1\) Student’s t-test or the chi-square test \(^2\)BMI: body mass index

Table 2 Mean intakes estimated from a self-administered diet-history questionnaire and mean serum concentrations

|                      | All participants \((n = 167)\) | S Hospital \((n = 76)\) | T Hospital \((n = 91)\) | \(p^1\) |
|----------------------|---------------------------------|------------------------|------------------------|-------|
|                      | Mean ± SD | Mean ± SD | Mean ± SD |       |
| Intakes              |          |          |          |       |
| Energy [kcal/day]    | 1823 ± 373 | 1832 ± 377 | 1815 ± 372 | 0.770 |
| Folate [µg/day]\(^2\) | 261 ± 107 | 215 ± 76 | 299 ± 114 | < 0.001 |
|                      | [µg/1,000 kcal]\(^2\) | 142 ± 48 | 117 ± 30 | 164 ± 50 | < 0.001 |
| Vitamin B\(_12\) [µg/day]\(^2\) | 5.74 ± 3.10 | 5.66 ± 3.54 | 5.80 ± 2.70 | 0.328 |
|                      | [µg/1,000 kcal]\(^2\) | 3.10 ± 1.35 | 3.01 ± 1.49 | 3.17 ± 1.22 | 0.200 |
| Serum concentrations  |          |          |          |       |
| Folate [ng/ml]\(^2\) | 657 ± 280 | 525 ± 252 | 767 ± 255 | < 0.001 |
| Vitamin B\(_12\) [pg/ml]\(^2\) | 268 ± 99 | 199 ± 69 | 325 ± 83 | < 0.001 |

\(^1\) Student’s t-test \(^2\) The analyses were conducted after log-transformation of the variables
0.222 to 0.313, whereas the correlation coefficient for folate decreased slightly. The mean dietary intakes in quintiles based on serum concentrations are presented in Table 4. When participants were classified into quintiles based on intakes and serum concentrations of folate and vitamin B₁₂, approximately 60% fell into the same or adjacent quintiles. Meanwhile, 4-5% of the participants were classified in the opposite quintile.

Reproducibility study
A total of 64 pregnant women in the T hospital completed two DHQ rounds. Of these, we excluded 6 pregnant women from the analysis: 3 dropped out, 1 had missing data, and 2 had a severe under-reported energy intake. Ultimately, data of 58 women were analyzed to assess the DHQ reproducibility. Of the 58 participants, 13 were included in the validation study. There were no significant differences in the characteristics between participants in the reproducibility study and T hospital participants in the validation study (Table 1).

No significant differences were found in the mean intakes of folate and vitamin B₁₂ estimated from the two-time DHQ (Table 5). Intraclass correlation coefficients of daily intakes between two-time DHQ were 0.725 for folate and 0.512 for vitamin B₁₂. When each intake estimated from the two-time DHQ was classified into quintiles, more than 70% of the pregnant women were classified in the same or adjacent quintiles. The percentage of pregnant women classified into the opposite quintile was 0% for folate and 1.7% for vitamin B₁₂ (Table 6).

Table 3 Correlation coefficients between intakes and serum concentrations of folate and vitamin B₁₂

| Intakes³ | All participants (n = 167) | Participants without nausea (n = 121)² |
|----------|---------------------------|--------------------------------------|
|          | r | p¹ | r | p¹  |
| Folate [μg/day] | 0.251 | 0.001 | 0.245 | 0.007 |
| [μg/1,000 kcal] | 0.286 | < 0.001 | 0.280 | 0.002 |
| Vitamin B₁₂ [μg/day] | 0.174 | 0.025 | 0.249 | 0.006 |
| [μg/1,000 kcal] | 0.222 | 0.004 | 0.313 | 0.001 |

¹Partial correlation analysis. Adjusted for research settings. All variables were log-transformed before analyses. ²The participants with nausea were excluded from the analyses. ³Folate and vitamin B₁₂ intakes were assessed by a self-administered diet history questionnaire (DHQ).

Table 4 Mean intakes in each quintile based on corresponding serum concentrations and cross-classification between quintiles of intakes and serum concentrations (n = 167)

| Corresponding serum concentrations | Agreement (% classified into the same or adjacent quintile)¹ | Discordance (% classified into the opposite quintile)¹ | Linear trend² p |
|-----------------------------------|----------------------------------------------------------|-----------------------------------------------------|-----------------|
| 1st quintile | 2nd quintile | 3rd quintile | 4th quintile | 5th quintile |
| Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD |
| Intakes | | | | |
| Folate [μg/day] | 209 ± 96 | 258 ± 91 | 249 ± 91 | 286 ± 101 | 296 ± 134 | 64.7 | 4.8 | 0.003 |
| [μg/1,000 kcal] | 115 ± 39 | 145 ± 46 | 139 ± 49 | 145 ± 35 | 164 ± 58 | 61.1 | 3.6 | 0.002 |
| Vitamin B₁₂ [μg/day] | 4.62 ± 2.43 | 6.36 ± 4.20 | 6.02 ± 4.9 | 4.88 ± 2.91 | 6.82 ± 1.81 | 54.5 | 4.2 | 0.029 |
| [μg/1,000 kcal] | 2.57 ± 1.29 | 3.23 ± 1.68 | 3.20 ± 1.24 | 2.90 ± 0.84 | 3.58 ± 1.41 | 59.9 | 4.2 | 0.005 |

¹Agreement and discordance in quintile ranking was assessed as the percentage classification for the pregnant women based on intake and corresponding serum concentration in the same, adjacent, and opposite quintiles. ²Linear regression analyses were performed to test for significant linear trends between intakes and serum concentrations, adjusting for research settings. The dependent variables were log-transformed.

Folate and vitamin B₁₂ intakes were assessed by a self-administered diet history questionnaire (DHQ).
The Bland-Altman plots of folate and vitamin B₁₂ showed that most participants were in the accepted range of agreement, but a few women showed differences between the intakes estimated from the two-time DHQ beyond the accepted ranges (Figure 1 and 2).

Discussion
To our knowledge, this is the first study to validate the dietary intake of folate and vitamin B₁₂ in Japanese pregnant women using quantitative biological markers. Our findings showed correlation coefficients of 0.222-0.313 between energy-adjusted intakes and serum concentrations of folate and vitamin B₁₂. In previous studies of non-pregnant women, the reported correlation coefficients ranged from 0.20 to 0.51 for folate and from 0.20 to 0.27 for vitamin B₁₂[18-21]. Meanwhile, a Norwegian study of pregnant women found a correlation coefficient of 0.26 for folate between intakes and serum concentrations [22]. In general, correlation coefficients of more than 0.50 are considered as closely correlated, 0.30-0.50 as acceptable, and less than 0.30 as poorly correlated [23]. However, the correlations between intakes and biomarkers are often lower during pregnancy than in the non-pregnant period. This is because some nutrients are required for continuation of pregnancy and fetal development, and a greater intrindividual variability in intakes can occur during pregnancy than non-pregnancy periods [12,24,25]. Compared with the criteria of Ortiz-Andrellucchi et al. and another study on pregnancy [22,23], the present study showed acceptable validity for assessing energy-adjusted intakes of folate and vitamin B₁₂. Using the energy-adjusted values is recommended in epidemiological studies because energy adjustment can reduce intraindividual measurement errors [26]. Therefore, the DHQ can be used in epidemiological studies for Japanese pregnant women.

The correlation coefficient between the intake and serum concentration of vitamin B₁₂ increased in participants without nausea in the present study. Nausea and vomiting in pregnancy often alter food consumption, and a previous study showed that pregnant women with nausea consumed less meat products, which contain large amount of vitamin B₁₂, than those without nausea, whereas consumption of vegetables and fruits, which contain large amount of folate, were not affected by nausea [27]. Since the degree of nausea varies daily and

| Table 5 Mean daily intakes and Intraclass Correlation Coefficients between intakes estimated from the two-time DHQ (n = 58) |
|---------------------------------------------------------------|
| 1st DHQ | 2nd DHQ | 1st DHQ + 2nd DHQ Intraclass Correlation Coefficient |
| Mean ± SD | Mean ± SD | p |  |
| Energy [kcal/day] | 1727 ± 407 | 1771 ± 399 | 0.523 | 0.599 |
| Folate [μg/day]² | 292 ± 120 | 279 ± 112 | 0.257 | 0.725 |
| Vitamin B₁₂ [μg/day]² | 5.37 ± 2.79 | 5.91 ± 2.95 | 0.052 | 0.512 |

DHQ: self-administered diet history questionnaire
²The analyses were performed after log-transformation of the variables to achieve normal distribution

| Table 6 Mean intakes estimated from the 2nd DHQ across quintiles of intakes estimated from the 1st DHQ (n = 58) |
|---------------------------------------------------------------|
| 1st DHQ | Agreement (% classified into the same or adjacent quintile)¹ | Discordance (% classified into the opposite quintile)¹ | Linear trend² | p |
| ¹ | ² | ³ | ¹ | ² | ³ | p |
| 1st quintile | 2nd quintile | 3rd quintile | 4th quintile | 5th quintile | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD |
| Energy [kcal/day] | 1452 ± 498 | 1786 ± 292 | 1591 ± 227 | 1908 ± 300 | 2120 ± 323 | 70.7 | 1.7 | < 0.001 |
| Folate [μg/day]³ | 185 ± 44 | 241 ± 37 | 243 ± 60 | 288 ± 63 | 447 ± 126 | 81.1 | 0 | < 0.001 |
| Vitamin B₁₂ [μg/day]³ | 4.44 ± 3.2 | 5.03 ± 3.7 | 5.27 ± 6.0 | 6.16 ± 6.3 | 8.77 ± 4.3 | 77.6 | 1.7 | < 0.001 |

DHQ: self-administered diet history questionnaire
¹Agreement and discordance in quintile ranking was assessed as the percentage classification for the pregnant women based on the two-time DHQ in the same, adjacent, and opposite quintiles
²Linear regression analyses were performed to test for significant linear trends between estimated intakes from the two-time DHQ
³Linear regression analyses were conducted after log-transformation of the variables
as gestation progresses, food selections would change over time. Therefore, among pregnant women with nausea, assessing habitual consumption of foods that can be affected by nausea might be difficult. In the present study, the correlation coefficient and the cross-classification analysis showed that the DHQ provided a reasonable validity for assessing energy-adjusted intakes of vitamin B$_{12}$, regardless of the possibility of nausea. However, habitual vitamin B$_{12}$ intakes estimated from the DHQ in pregnant women with nausea need to be interpreted carefully because the correlation coefficient of vitamin B$_{12}$ in all participants including pregnant women with nausea was lower than the acceptable level of 0.30 [23].
In the present study, we excluded pregnant women taking supplements including folic acid and vitamin B12, which was 49% of the recruited pregnant women in the T hospital. This percentage in the second trimester was twice as high as that in the S hospital and in other studies of Japanese pregnant women [28,29]. The attitudes toward dietary intakes and supplementation might have been affected by the difference between hospitals. However, serum concentrations of folate and vitamin B12 in the present study were similar to other studies among non-supplement users [30,31]. Therefore, we considered that our participants were representative of Japanese pregnant women in the general population in terms of folate and vitamin B12 status.

Good correlation coefficients achieved in the reproducibility study of the dietary assessment questionnaire range from 0.50 to 0.70 [32]. Since the correlation coefficient for folate and vitamin B12 was 0.725 and 0.512, respectively, in the present study, the intakes estimated from the two-time DHQ showed good reproducibility among pregnant women. Meanwhile, mean vitamin B12 intake from the first DHQ was lower than that from the second DHQ, although non-significantly. In addition, the Bland-Altman plots showed an unacceptable difference between the two-time DHQ in a few pregnant women. We speculate that this result for vitamin B12 might be partly due to the altered food selection associated with nausea and vomiting, as discussed for the validation study [27]. Even if participants had no nausea or vomiting when completing the DHQ, some might have had such symptoms in the object period of the first DHQ because the period included 11-12 gestation weeks when most pregnant women suffer nausea and vomiting [33]. On the other hand, cross-classification analyses and linear trends indicated that the DHQ could determine pregnant women with low and high intakes of both folate and vitamin B12. The fact that the reproducibility of the DHQ during pregnancy was established even in such a slightly unstable period indicated that the DHQ is probably a reliable dietary assessment tool throughout pregnancy.

The current study had several limitations. First, the characteristics of the participants were likely to be biased because one of the research hospitals was a university hospital in an urban area. Second, prepregnancy weight and height were self-reported. Therefore, the reporting bias might affect the values. Third, we did not test for 5,10-methyltetrahydrofolate reductase (MTHFR) genotypes, which affects folate metabolism. Thirteen to sixteen percent of Japanese people have the TT genotype, with lower circulating folate levels [34,35]. Therefore, the relationship between intake and serum concentration of folate might vary depending on MTHFR genotype. Fourth, we could not obtain detailed information regarding intakes of fortified foods with folic acid. In Japan, a few food products such as sweets, eggs, milk, and candy are fortified with folic acid. Accordingly, this might have affected the result of the present study. Finally, full diet composition might not be estimated from the DHQ due to variations in capacity of the described food and seasonal change.

**Conclusion**

The present study confirmed that the DHQ had acceptable validity and reproducibility for assessing folate and vitamin B12 intakes in Japanese pregnant women. This result would be useful for nutritional intervention studies and epidemiological studies of the relationship between pregnancy complications and intake of folate and vitamin B12 in Japan.

**Abbreviations**

DHQ: Self-administered diet history questionnaire; BMI: Body mass index; MTHFR: Methylenetetrahydrofolate reductase.

**Acknowledgements**

This work was supported by Institute for Food and Health Science, Yazuya Co Ltd, 2009 and Fumiko Yamaji Trust for Academic Nursing Education and Research, 2011. We are deeply grateful to the participants and the hospital staff for their cooperation.

**Author details**

1. Department of Midwifery and Women’s Health, Division of Health Sciences and Nursing, Graduate School of Medicine, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. 2. Department of Social and Preventive Epidemiology, School of Public Health, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. 3. Department of Community Health Nursing, Division of Health Sciences and Nursing, Graduate School of Medicine, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.

**Authors’ contributions**

All authors conceived and designed the study. MS performed the statistical analyses. MS, MH, MM and SS interpreted the results. MS drafted the manuscript. All authors revised the manuscript for intellectual content, and read and approved the final manuscript.

**Competing interests**

The authors declare that they have no competing interests.

Received: 26 October 2011 Accepted: 15 March 2012

**Published: 15 March 2012**

**References**

1. Barker DJ, Osmond C, Golding J, Kuh D, Wadsworth ME: Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. BMJ 1989, 298:564-567.
2. Ministry of Health, Labour and Welfare. Population Survey Report. 2010 [http://www.mhlw.go.jp/toukei/saikin/hw/jinkou/tokusyu/syussyo06/ syussyo2.html#05].
3. Takimoto H, Yokoyama T, Yoshiike N, Fukuoka H: Increase in low-birthweight infants in Japan and associated risk factors, 1980-2000. J Obstet Gynaecol Res 2005, 31:314-322.
4. Takimoto H, Yoshiike N, Katagiri A, Ishida H, Abe S: Nutritional status of pregnant and lactating women in Japan: a comparison with non-pregnant/non-lactating controls in the National Nutrition Survey. J Obstet Gynaecol Res 2003, 29:95-103.
5. Vollset SE, Refsum H, Legens LM, Emblem BM, Tverdal A, Gjessing HK, Monsen AL, Ueland PM: Plasma total homocysteine, pregnancy.
