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Maternal intake of one-carbon metabolism-related B vitamins and anorectal malformations in the Japan Environment and Children's Study

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Abstract

The occurrence of anorectal malformations (ARM) is thought to be reduced with sufficient folate intake. However, there is no apparent evidence. We focused on enzyme cofactors for one-carbon metabolism, including folate (vitamin B_9), vitamin B_6 and vitamin B_{12}, and explored the association between maternal combined intake of these B vitamins and the risk of ARM. Using baseline data from a Japanese nationwide birth cohort study between 2011 and 2014, we analysed data of 89 235 women (mean age at delivery = 31·2 years) who delivered singleton live births without chromosomal anomalies. Information on dietary intake was obtained via a FFQ focused on early pregnancy and used to estimate B vitamin intake. We also collected information on the frequency of folic acid supplement use. ARM occurrence was ascertained from medical records. We identified forty-three cases of ARM diagnosed up to the first month after birth (4·8 per 10 000 live births). In terms of individual intake of the respective B vitamins, high vitamin B_6 intake was non-significantly associated with reduced odds of ARM. Compared with women in the low combined B vitamin intake group, the OR of having an infant with ARM was 0·4 (95 % CI 0·2, 1·0) in the high intake group (folate ≥400 µg/d, and upper half of vitamin B_6 and/or vitamin B_{12}). In conclusion, our cohort analysis suggested an inverse association between the combined intake of one-carbon metabolism-related B vitamins in early pregnancy and ARM occurrence.

Key words: **Folate: Vitamin B_6: Vitamin B_{12}: Anorectal malformations: Birth cohorts**

Anorectal malformations (ARM) refer to atresia or stenosis of the anus and rectum, with or without a fistula between the bladder, urethra, perineum or vestibule[1]. The frequency of ARM appears to be roughly 4 per 10 000 births, though the frequency differs somewhat by country[2–4]. In 2013, the mortality from ARM in Japan was the lowest among the neonatal surgical diseases[5]. Data from the North-East Italy Registry showed that the 10-year survival probability for isolated ARM was 100 %[6]. However, ARM require surgical repair, and infants with ARM may suffer from perioperative complications and typically repeated outpatient visits after discharge. Moreover, since parents know that their infants need surgical intervention in the first year of life, they often face lasting distress and anxiety and become exhausted with the long-term care of their infants[7]. The patients themselves often suffer from active long-term problems, such as faecal incontinence, chronic constipation, urinary incontinence, ejaculatory dysfunction and/or erectile dysfunction[8]. Further, from the viewpoint of medical economics, it is impossible to overlook the financial burden of ARM, even if the condition is uncommon. In the USA, for example, the average total hospital charges for the primary repair of ARM in 2012 were estimated to be $72 631 per hospital stay[9]. Thus, we should consider the possibility of primary prevention of ARM.

Insufficient maternal intake of folate is an established risk factor for neural tube defects[10,11]. To prevent folate insufficiency among childbearing-aged women, staple foods have been fortified with folic acid in many countries and nationwide folic acid fortification resulted in a decline in the occurrence of

Abbreviations: ARM, anorectal malformation; JECS, Japan Environment and Children's Study.

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neural tube defects[12,13]. Interestingly, such fortification was likely to have also reduced the occurrence of non-neural tube defect anomalies[12], and ARM may have been one of these latter types of anomaly. In a public health campaign conducted in China between 1993 and 1995, use of a 400-μg folic acid supplement was associated with a roughly 40 % decrease in the risk of ARM when compared with non-use[14]. Also, an experimental study reported that Adriamycin-induced ARM occurrence decreased in rats supplemented with folic acid during pregnancy[15]. However, meta-analysis based on the results of seven epidemiological studies indicated that maternal use of folic acid supplement was not associated with ARM (OR 0·93, 95 % CI 0·77, 1·13)[16]. In contrast, the use of vitamins or supplements that containing folic acid was associated with a reduced risk of ARM among women without diabetes mellitus, in a US multicentre case–control study[17], suggesting possible limitations in evaluating the independent effects of folate on ARM. Folate (vitamin B_9) has a major role in the one-carbon metabolism related to the synthesis of purine and thymidine nucleotides, and the synthesis of methionine from homocysteine[18]. Likewise, vitamins B_6 and B_{12} act as enzyme cofactors for one-carbon metabolism[11,19]; for example, vitamin B_6 is a cofactor in the conversion of tetrahydrofolate to 5,10-methylene tetrahydrofolate, and vitamin B_{12} works a cofactor in the conversion of homocysteine and 5-methyltetrahydrofolate to methionine and tetrahydrofolate, respectively. Thus, evaluation not merely of folate alone, but of the combination of vitamins B_6, B_9 and B_{12}, appears likely to provide substantive new findings regarding the association between folate intake and ARM.

The purpose of this study was to explore the extent to which maternal intake of one-carbon metabolism-related B vitamins, including folate, vitamin B_6 and vitamin B_{12}, was associated with the risk of ARM. We hypothesised that a high combined intake of these three nutrients reduces the occurrence of ARM.

Methods

Study participants

The Japan Environment and Children's Study (JECS), an ongoing nationwide birth cohort study, includes data on 103 099 pregnancies. From 2011 to 2014, women were recruited as early in pregnancy as possible (median = 12th week of gestation, interquartile range = 10, 15th) in fifteen Regional Centres throughout Japan. The concept and design have been previously described in detail[20]. The respective distributions of maternal and infant characteristics in the JECS were comparable with those obtained in the national survey[21]. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving participants were approved by the Japan Ministry of the Environment's Institutional Review Board on Epidemiological Studies (No. 100406001) and the Ethics Committees of all participating institutions. Written informed consent was obtained from all participants.

Of the 103 099 pregnancies, 95 170 unique mothers (excluding repeated registration) with subsequent delivery record were identified. In this study, we excluded participants who delivered infants with Down's syndrome, trisomy 18 or trisomy 13, to exclude cases of ARM related to chromosomal factors[22] (further exclusions are summarised in online Supplementary Fig. S1), resulting in 89 235 mothers who delivered singleton live births being included in the analysis.

Dietary assessment

We distributed self-administered questionnaires twice, first during the first trimester (median fill-in week of gestation = 15), and then again during the second/third trimester (27th week of gestation). The first questionnaire included a FFQ regarding dietary intake in the preceding year, and the second a FFQ regarding the same items, but focusing on usual intake after awareness of pregnancy. In this study, we used data from the first FFQ as a marker of dietary intake in early pregnancy and treated it as a main exposure index reflecting exposure during the ARM developmental origin period. We used data from the second FFQ as a marker for mid-late pregnancy.

The FFQ used in the maternal surveys was developed for the Japan Public Health Centre-based prospective Study for the Next Generation and validated for 142 Japanese women aged 40–74 years, using a 12-d weighed food record[23]. This FFQ focused on the frequency of consumption and portion size for each food item. The response choices for frequency were: <1 time/month, 1–3 times/month, 1–2 times/week, 3–4 times/week, 5–6 times/week, 1 time/d, 2–3 times/d, 4–6 times/d and ≥7 times/d; and those for portion size were: small (50 % less than standard), medium (equal to standard) and large (50 % more than standard). We calculated the daily food intake by multiplying the frequency by the standard-equivalent portion size, for each food item. The daily intake of nutrients, such as folate, vitamin B_6 and vitamin B_{12}, was estimated using the Standard Tables of Food Composition in Japan 2010[24]. We adjusted for total energy intake using the residual model[25].

The first and second questionnaires included a question about the frequency of taking folic acid supplement at the time, with seven response choices: none, once in a month, 2–3 times a month, 1–3 times a week, 4–6 times a week, once a day and twice or more times a day. We also collected information on the supplemental use of multi-vitamins (only information on use or non-use), including B vitamins, from preconception to 12 weeks of gestation, via face-to-face interviews. As there was a lack of information regarding amount of use, we did not include the nutrient intake from such supplements in the FFQ-based estimation. In Japan, there are no national food-fortification programmes involving folic acid.

Identification of anorectal malformations

Information on physician diagnoses of ARM was retrieved from the medical records. In accordance with the JECS in-house standard operating procedures, medical record transcriptions were performed three times by physicians, midwives/nurses and/or Research Coordinators: first during the first trimester, second after delivery and finally at the first-month health check-up after delivery. We used data from the forms after delivery and a month after delivery, which contained a list of sixty-one

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congenital anomalies, including ARM (10th edition of the International Classification of Diseases: Q42)[26], and registered ARM occurrence when ARM were reported in either form. Information on the clinical classification of the respective ARM was not collected.

Statistical analysis

Based on a large sample size, an exploratory analysis on an observational study was done. Therefore, we did not perform a power calculation.

Given the small number of cases, we categorised the participants into two groups (high or low) based on the dietary assessment information. In the case of folate, women who consumed the estimated average requirement of dietary folate in Japan (≥400 μg/d)[27], or reported daily use of a folic acid supplement (once a day or more), were assigned to the high intake group. In Japan, daily folic acid supplements, with 400 μg of the monoglutamate form of folate, are typically available[28]. In the case of dietary intake of vitamin B_6 and B_{12}, the median values were used for assignment to the high or low groups. Based on the B vitamin intake group data for early pregnancy, we summarised the following baseline characteristics of the 89 235 mothers: maternal age at delivery; smoking habits; alcohol consumption; pre-pregnancy BMI; current history of diabetes or gestational diabetes mellitus; infertility treatment; educational background; household income; occupation; use of multivitamin supplements; week of pregnancy at delivery; parity and infant sex.

The association of folate, vitamin B_6 and B_{12} intake in early pregnancy with ARM occurrence was examined using logistic regression models, and the OR and 95 % CI of ARM, with the low intake group as the reference, were estimated. The first model was adjusted for maternal age at delivery. The multivariable model was further adjusted for the suspected risk factors of ARM, including smoking habits, alcohol consumption, pre-pregnancy BMI, diabetes or gestational diabetes mellitus and infertility treatment[29,30]. In the case of B_6 and B_{12} intake, we additionally adjusted for use of folic acid supplement. To explore whether the combined intake of folate, vitamin B_6 and vitamin B_{12} was associated with ARM occurrence, we defined three groups: low combined intake as reference (folate <400 μg/d, and low vitamin B_6 and/or low vitamin B_{12}), high intake (folate ≥400 μg/d, and high vitamin B_6 and/or high vitamin B_{12}) and remainder.

Through several sensitivity analyses, we confirmed whether consistent findings were obtained. First, we further adjusted for parity, hypertensive disorders of pregnancy, epilepsy during pregnancy[31], and protein, fish and vegetable intake in early pregnancy as possible confounders. Fish intake, which was inversely associated with congenital gastrointestinal tract atresia (oesophageal atresia, intestinal atresia and ARM) in our previous study[32], was not included in the multivariable model because fish is a major food source of vitamin B_6 and B_{12} in Japan[33], and fish consumption in early pregnancy was correlated with the intake of these vitamins (Spearman's correlation coefficient (ρ): 0·54 for vitamin B_6 and 0·80 for vitamin B_{12}). Vegetable intake was treated as a marker of healthy dietary habit. Second, we additionally adjusted for socio-economic status. Third, to avoid exposure misclassification, we excluded users of multi-vitamin supplements and participants with severe morning sickness. Fourth, the analysis was performed only on isolated cases of ARM (ARM with no other major congenital anomalies[34,35]), to exclude the potential influence of genetic factors. Fifth, further adjustment for combined intake of folate, vitamin B_6 and vitamin B_{12} in mid-late pregnancy was conducted, to focus on the independent association between intake in early pregnancy and ARM.

Additionally, we repeated the above analyses, to examine the association of folate, vitamin B_6 and vitamin B_{12} intake in mid-late pregnancy with ARM occurrence. For this intake period, we excluded mothers who did not have data from the second FFQ and delivered their infants at 22–27 weeks of gestation, leaving a total of 88 234 mothers available for analysis. The present study used the data set jecs-ag-20160424, which was released in June 2016 and revised in October 2016, along with the supplementary data set jecs-ag-20160424-sp1. All analyses were performed with Stata 15 (StataCorp LP).

Results

Among the 89 235 participants (mean age at delivery = 31·2 years), forty-three women delivered infants with ARM (4·8 per 10 000 live births). Overall, the median dietary intakes in early pregnancy were 245·4 μg/d for folate (estimated average requirement in Japan: 400 μg/d), 1·0 mg/d for vitamin B_6 (1·2 mg/d) and 3·8 μg/d for vitamin B_{12} (2·3 μg/d); 28·3 % of the participants used a daily folic acid supplement in early pregnancy.

Table 1 shows the distribution of mothers' baseline characteristics for the folate, vitamin B_6 and vitamin B_{12} intake groups. In the case of folate, the high dietary and supplemental intake group showed a higher percentage of women with the following characteristics, compared with the low intake group: age at delivery ≥35 years, never-smoked, infertility treatment, educational background ≥13 years, household income ≥6 million Japanese-yen/year, use of multi-vitamin supplements and nulliparae. In online Supplementary Table S1, we summarise the baseline characteristics of the mothers who delivered infants with ARM.

Table 2 shows the association between the respective early pregnancy intakes of B vitamins and ARM. High vitamin B_6 intake was non-significantly associated with decreased odds of ARM occurrence (adjusted OR for high *v.* low group = 0·5, 95 % CI 0·3, 1·0). Likewise, the estimated OR for folate and vitamin B_{12} intake showed a direction of lower risk. In terms of the association between combined B vitamin intake and ARM, the adjusted OR in the high combined intake group was 0·4 (95 % CI 0·2, 1·0) compared with the low intake group (Table 3); and the estimated OR in this high group did not vary substantially in the various sensitivity analyses.

Table 4 shows the association between the respective mid-late pregnancy intakes of B vitamins and ARM. B vitamin intake in this period was moderately correlated with that in early pregnancy (ρ = 0·57 for folate, 0·58 for vitamin B_6 and 0·48 for

Table 1. Baseline characteristics of 89 235 pregnant women, in terms of maternal intake of folate, vitamin B_6 and vitamin B_{12} in early pregnancy, Japan Environment and Children's Study (2011–2014)
(Numbers and percentages; medians)

		Dietary and supplemental intake of folate		Dietary intake of vitamin B_6		Dietary intake of vitamin B_{12}	
	No. of women*	Low (<400 μg/d) (*n* 59 573) (%)	High (≥400 μg/d) (*n* 29 662) (%)	Low (<1·0 mg/d) (*n* 44 617) (%)	High (≥1·0 mg/d) (*n* 44 618) (%)	Low (<3·8 μg/d) (*n* 44 615) (%)	High (≥3·8 μg/d) (*n* 44 620) (%)
Dietary intake in early pregnancy							
Energy (kJ/d), median	89 235	7072	7097	6695	7348	6958	7176
Protein (g/d), median	89 235	57·1	59·1	53·4	61·9	53·1	62·2
Folate (μg/d), median	89 235	235·4	270·6	204·6	293·1	226·5	263·3
Vitamin B_6 (mg/d), median	89 235	1·0	1·1	0·9	1·1	0·9	1·1
Vitamin B_{12} (μg/d), median	89 235	3·8	4·0	3·1	4·7	2·7	5·2
Fish (g/d), median	89 235	31·2	33·2	23·0	42·6	19·8	47·1
Vegetables (g/d), median	89 235	147·8	181·5	117·3	207·4	145·0	168·9
Fruits (g/d), median	89 235	110·4	129·6	88·2	146·2	111·9	121·1
Use of a folic acid supplement in early pregnancy							
<1 time/d	63 969	100	14·8	74·2	69·2	72·6	70·8
≥1 time/d	25 266	0	85·2	25·8	30·9	27·4	29·2
Age at delivery (years)							
<25	8603	11·5	5·9	12·4	6·9	11·3	8·0
25–29	24 537	28·7	25·0	29·5	25·5	29·3	25·7
30–34	31 680	34·4	37·7	33·9	37·1	34·6	36·4
≥35	24 415	25·3	31·4	24·2	30·5	24·9	29·9
Smoking habits							
Never smoked	51 999	56·8	61·4	54·1	62·6	57·7	59·0
Ex-smokers/smokers	37 113	43·2	38·6	45·9	37·4	42·3	41·0
Alcohol consumption							
Never drank	30 713	34·9	33·6	35·7	33·2	35·8	33·1
Ex-drinkers/drinkers	58 505	65·1	66·5	64·3	66·8	64·2	66·9
Pre-pregnancy BMI							
<18·5 kg/m^2	14 406	16·0	16·6	16·8	15·5	16·6	15·7
18·5–24·9 kg/m^2	65 370	72·9	74·1	72·3	74·2	72·8	73·8
≥25·0 kg/m^2	9421	11·2	9·4	10·9	10·3	10·6	10·5
Current history of diabetes or gestational diabetes							
No	86 429	97·0	96·6	97·0	96·7	97·1	96·6
Yes	2806	3·0	3·5	3·0	3·3	2·9	3·4
Infertility treatment							
No	83 193	95·2	89·4	94·4	92·2	93·8	92·7
Yes	6015	4·8	10·6	5·7	7·8	6·2	7·3
Educational background (years)							
<13	31 496	38·7	30·0	42·0	29·6	38·4	33·2
≥13	56 490	61·3	70·0	58·0	70·4	61·6	66·8
Household income (million Japanese-yen/year)							
<6	60 086	75·1	69·0	76·2	69·9	75·3	70·8
≥6	22 192	24·9	31·1	23·8	30·1	24·7	29·2
Occupation in early pregnancy							
Administrative, managerial, professional or engineering	20 568	22·8	24·1	21·1	25·4	22·9	23·6
Clerical	15 224	16·5	18·6	17·1	17·3	17·1	17·3
Sales and service	19 438	23·4	19·0	24·9	19·0	23·3	20·6
Homemaker	24 686	27·0	29·6	26·1	29·6	26·4	29·3
Other	8657	10·3	8·7	10·8	8·8	10·4	9·2
Use of multi-vitamin supplements							
No	82 059	94·7	87·6	93·0	91·7	92·7	92·0
Yes	6797	5·3	12·4	7·0	8·3	7·3	8·0
Week of pregnancy at delivery							
<37 weeks (preterm)	4143	4·6	4·8	4·7	4·6	4·7	4·6
≥37 weeks	85 092	95·4	95·2	95·3	95·4	95·3	95·4
Parity							
0	38 938	39·7	51·9	46·2	41·3	46·4	41·2
≥1	49 995	60·3	48·1	53·8	58·7	53·6	58·8
Infant sex							
Boys	45 808	51·2	51·7	51·5	51·2	51·5	51·2
Girls	43 419	48·8	48·3	48·5	48·8	48·5	48·8

* Subgroup totals do not equal the overall number because of missing data.

Table 2. Anorectal malformation, in terms of maternal intake of folate, vitamin B_6 and vitamin B_{12} in early pregnancy, Japan Environment and Children's Study (2011–2014)
(Odds ratios and 95 % confidence intervals)

	No. of women	No. of cases	Maternal age-adjusted model			Multivariable model 1*			Multivariable model 2*†		
			OR	95 % CI	*P*	OR	95 % CI	*P*	OR	95 % CI	*P*
Dietary and supplemental intake of folate (μg/d)											
Low (<400 μg/d)	59 573	29	1·0			1·0			–	–	
High (≥400 μg/d)	29 662	14	1·0	0·5, 1·8	0·89	0·9	0·4, 1·6	0·65	–	–	
Dietary intake of vitamin B_6 (mg/d)											
Low	44 617	27	1·0			1·0			1·0		
High	44 618	16	0·6	0·3, 1·1	0·09	0·5	0·3, 1·0	0·06	0·5	0·3, 1·0	0·06
Dietary intake of vitamin B_{12} (μg/d)											
Low	44 615	25	1·0			1·0			1·0		
High	44 620	18	0·7	0·4, 1·3	0·27	0·7	0·4, 1·3	0·25	0·7	0·4, 1·3	0·25

* OR were estimated using logistic regression model that included maternal age at delivery, smoking habits, alcohol consumption, pre-pregnancy BMI, current history of diabetes or gestational diabetes and infertility treatment. Participants with missing values for these factors were excluded, which left 89 034 in the multivariable models.
† Additionally adjusted for use of folic acid supplement in early pregnancy.

Table 3. Association between combined intake of folate, vitamin B_6 and vitamin B_{12} in early pregnancy and anorectal malformation (ARM)
(Odds ratios and 95 % confidence intervals)

	Combined intake of folate, vitamin B_6 and vitamin B_{12}: Low (folate <400 μg/d, and low vitamin B_6 and/or low vitamin B_{12})	Remainder			High (folate ≥400 μg/d, and high vitamin B_6 and/or high vitamin B_{12})		
		OR	95 % CI	*P*	OR	95 % CI	*P*
Main analysis							
No. of women	40 965	26 565			21 705		
No. of cases	25	11			7		
Maternal age-adjusted model	1·0	0·7	0·3, 1·4	0·26	0·5	0·2, 1·2	0·12
Multivariable model*	1·0	0·6	0·3, 1·3	0·21	0·4	0·2, 1·0	0·06
Sensitivity analyses							
Additionally adjusted for parity, hypertensive disorders of pregnancy, epilepsy during pregnancy, and protein, fish and vegetable intake in early pregnancy							
No. of women	40 965	26 565			21 705		
No. of cases	25	11			7		
Multivariable model*	1·0	0·7	0·3, 1·6	0·42	0·5	0·2, 1·2	0·13
Additionally adjusted for socio-economic status, including educational background, household income and occupation in early pregnancy							
No. of women	37 067	24 464			20 127		
No. of cases	23	10			7		
Multivariable model*	1·0	0·6	0·3, 1·4	0·25	0·5	0·2, 1·2	0·13
Excluding users of multi-vitamin supplements							
No. of women	38 688	24 450			18 921		
No. of cases	25	9			7		
Multivariable model*	1·0	0·5	0·3, 1·2	0·11	0·5	0·2, 1·2	0·11
Excluding participants with severe morning sickness							
No. of women	36 171	23 753			19 669		
No. of cases	24	9			7		
Multivariable model*	1·0	0·5	0·2, 1·2	0·12	0·5	0·2, 1·1	0·08
Isolated cases of ARM (cases without other major anomalies†)							
No. of women	40 957	26 564			21 704		
No. of cases	17	10			6		
Multivariable model*	1·0	0·9	0·4, 1·9	0·69	0·6	0·2, 1·5	0·26
Additionally adjusted for combined intake of folate, vitamin B_6 and vitamin B_{12} in mid-late pregnancy							
No. of women	40 461	26 276			21 497		
No. of cases	25	11			7		
Multivariable model*	1·0	0·6	0·3, 1·2	0·14	0·4	0·2, 1·0	0·05

* OR were estimated using logistic regression model that included maternal age at delivery, smoking habits, alcohol consumption, pre-pregnancy BMI, current history of diabetes or gestational diabetes and infertility treatment.
† Major anomalies included anencephaly, spina bifida, encephalocele, microphthalmia, cleft palate, cleft lip (with or without cleft palate), congenital heart disease (not including patent ductus arteriosus), gastroschisis, omphalocele, diaphragmatic hernia, oesophageal atresia, small intestinal atresia, hypospadias and reduction defects of the upper and/or lower limbs[34,35].

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Table 4. Anorectal malformation, in terms of maternal intake of folate, vitamin B_6 and vitamin B_{12} in mid-late pregnancy, Japan Environment and Children's Study (2011–2014)*
(Odds ratios and 95 % confidence intervals)

	No. of women	No. of cases	Maternal age-adjusted model			Multivariable model 1†			Multivariable model 2		
			OR	95 % CI	*P*	OR	95 % CI	*P*	OR	95 % CI	*P*
Dietary and supplemental intake of folate (μg/d)											
Low (<400 μg/d)	66 297	31	1·0			1·0			–	–	
High (≥400 μg/d)	21 937	12	1·2	0·6, 2·3	0·68	1·0	0·5, 2·0	0·91	–	– –	
Dietary intake of vitamin B_6 (mg/d)											
Low	44 124	25	1·0			1·0			1·0		
High	44 110	18	0·7	0·4, 1·3	0·27	0·7	0·4, 1·2	0·19	0·7‡	0·4, 1·2	0·19
Dietary intake of vitamin B_{12} (μg/d)											
Low	44 108	20	1·0			1·0			1·0		
High	44 126	23	1·1	0·6, 2·1	0·68	1·1	0·6, 2·0	0·72	1·1‡	0·6, 2·0	0·73
Combined intake of folate, vitamin B_6 and vitamin B_{12}											
Low (folate <400 μg/d, and low vitamin B_6 and/or low vitamin B_{12})	44 866	21	1·0			1·0			1·0		
Remainder	27 219	15	1·2	0·6, 2·3	0·66	1·1	0·6, 2·2	0·75	1·4§	0·7, 2·8	0·35
High (folate ≥400 μg/d, and high vitamin B_6 and/or high vitamin B_{12})	16 149	7	0·9	0·4, 2·1	0·82	0·8	0·3, 1·9	0·63	1·3§	0·5, 3·3	0·62

* We included 88 234 women who had valid data on a FFQ during the second/third trimester and delivered their infants after 28 weeks of gestation. OR were estimated using logistic regression model.
† Adjusted for maternal age at delivery, smoking habits, alcohol consumption, pre-pregnancy BMI, current history of diabetes or gestational diabetes and infertility treatment. Participants with missing values for these factors were excluded, which left 88 049 in the multivariable model.
‡ Additionally adjusted for use of folic acid supplement in mid-late pregnancy.
§ Additionally adjusted for combined intake of folate, vitamin B_6 and vitamin B_{12} in early pregnancy.

vitamin B_{12}) (online Supplementary Table S2). However, there was no association observed between ARM and B vitamin intake, either individually or in combination, in this period.

Discussion

In this prospective analysis of mothers recruited throughout Japan, the combined intake of folate, vitamin B_6 and vitamin B_{12} (rather than the individual intake of any one of these) appeared to be associated with reduced risk of their infants having ARM. This association, however, was only observed in early pregnancy, not in mid-late pregnancy. Given that the embryonic period (the first 8 weeks) is important in terms of the developmental origins of ARM[36], the observed tendency towards an inverse association with combined B vitamin intake in early pregnancy did not conflict with our hypothesis.

The JECS includes one of the largest birth cohorts in the world[21]. The frequency of ARM in the JECS population (4·8 per 10 000 live births) was comparable to past reports[2–4]; however, the number of outcome cases (forty-three) was small from the viewpoint of statistical rigour, and the possibility of chance findings remains. However, we should not underestimate the strength of the association; the estimated OR was 0·4 among mothers in the high combined intake group, compared with those in the low intake group. In this case, the E-value, which indicates the robustness of a given association with respect to potential unmeasured confounders[37], was 4·4. Thus, a confounder that was associated with B vitamin intake or ARM occurrence with a more than 4·4-fold relative risk might explain away the association here observed between B vitamins and ARM. One of the unmeasured possible confounders is infection-related fever during pregnancy; however, a large European case–control study reported that the adjusted OR for fever during the first 4 months of pregnancy was 2·2 (95 % CI 0·8, 5·7)[31]. Although, given the nature of observational investigation, the present study also had unmeasured confounders, the existence of such a significant confounder seems unlikely. With respect to measurement error which the E-value cannot evaluate, such error would naturally occur in dietary assessment based on self-reporting, and in the use of a FFQ not validated specifically for pregnant women, both of which apply to this study. However, such error would likely have here led to non-differential misclassification that would have weighted the OR point estimates towards null values, whereas an inverse association was suggested. Further, the estimated B vitamin intakes for the studied population did not deviate from the overall national estimate for women (median = 253 μg/d for folate, 0·97 mg/d for vitamin B_6 and 3·7 μg/d for vitamin B_{12} in the National Health and Nutrition Survey 2013[38]). Based on careful interpretation of the results, we concluded that there was a meaningful association between combined B vitamin intake and the occurrence of ARM.

A study in China between 1993 and 1995 reported a marginally decreased risk of ARM with regular folic acid supplementation (400 μg/d)[14], and a population-based case–control study in Hungary showed evidence of a preventive effect against ARM, through maternal folic acid supplementation[39]. However, no association between the use of folic acid and ARM was found in a number of observational studies[40–43], and dietary and supplemental folate intake was not associated with ARM occurrence in the present study. We had a related interest in the difference in ARM occurrence with the intake of multi-vitamins containing and not containing folic acid. A Netherlands case–control study based on 371 cases found no association with folic acid use (only 22 % of users also took multi-vitamins)[41], whereas a US case–control study based on 511 cases reported that intake of vitamins or supplements with folic acid was associated with reduced ARM risk among non-diabetic women[17]. This suggests the possibility there is a combined effect of folate and its related nutrients on ARM pathogenesis, rather than folate alone. We thus hypothesised that the disturbance of folate (or 'one-carbon') metabolism, which has been suggested as the pathogenesis of neural tube defects[18], had a role in ARM occurrence. Supporting the hypothesis of the present study, the study results suggested that combined intake of folate, vitamin B_6 and vitamin B_{12} tended to be protectively associated with ARM occurrence. There is evidence that supplements combining folic acid and vitamins B_6 and B_{12} reduced the homocysteine plasma level[44]. Given a low intake of B vitamins, impairment of one-carbon metabolism leads to homocysteine accumulation, which increases oxidative stress[18,45] and impairs nucleotide biosynthesis. These may inhibit the development of hindgut (the origin of the lower gastrointestinal tract). Certainly, oxidative stress seems to be involved in the pathogenesis of obesity- and diabetes-related congenital anomalies, including ARM[30,46]. However, no interaction was observed between folic acid use and methylenetetrahydrofolate reductase *C677T* polymorphism, in association with ARM[41]. The one-carbon metabolism pathway is a candidate for the underlying ARM mechanism, but would only partially account for the ARM pathogenesis. Since the pathogenesis of ARM is unclear, further studies, which focus on one-carbon metabolism, may contribute to its elucidation.

To the authors' knowledge, no birth cohort study has reported on differences in ARM occurrence between mothers who had high or low combined B vitamin intake. In addition, we used a nationwide cohort assumed to be representative of Japanese pregnant women[21], whose frequency of preconceptional folic acid use is typically lower than that of women in western countries[47]. Further, since we prospectively collected information on nutritional intake during pregnancy, we could ignore differences in the degree of recall bias between mothers who delivered infants with and without ARM, and the temporal association between exposure and outcome was warranted. Despite these strengths, we again note the limitation represented by the small number of ARM cases, which resulted in a broad 95 % CI for the OR point estimates, and should acknowledge some additional limitations of this study. One lay in the supposition that the women's usual dietary habits continued in early pregnancy. Since the embryonic period overlaps with the period of morning sickness, one's dietary habits might change in early pregnancy. Thus, we performed a sensitivity analysis that excluded participants with severe morning sickness and confirmed that this factor did not substantially affect the result. Another limitation is that we did not assess dietary methionine intake (a regulator of one-carbon metabolism). If methionine intake had been included in the analysis, it might have enabled more in-depth discussion of the contribution of one-carbon metabolism to ARM pathogenesis. Thirdly, we did not have information on ARM diagnosed after the first month after birth; however, most ARM cases are diagnosed during the newborn period[48]. Also, we did not collect information on the clinical classification of ARM. Finally, our analysis was restricted to women who delivered live births. This restriction, however, was unlikely to affect the target association because the majority of stillbirths with ARM appears to be caused by genetic and/or chromosomal factors.

In conclusion, we observed a suggestive inverse association between intake of one-carbon metabolism-related B vitamins in early pregnancy and ARM occurrence. Future research, focused on one-carbon metabolism, seems likely to contribute to elucidation of the pathogenesis of ARM.

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T. M., H. N. and S. Y. designed this study; T. M., H. N., M. S. and S. Y. contributed to the data analysis; T. M., H. N., S. F. N., T. I., E. S. and T. Kawamoto contributed to the data collection; T. M. wrote the initial draft of the manuscript; T. Kuroda and T. Kawamoto provided study supervision; All authors contributed to the interpretation of data, provided critical revisions of the manuscript and approved submission of the final manuscript.

None of the authors has any conflicts of interest to declare.

Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114520001816

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