British Journal of Nutrition (2022), 128, 2480–2489 doi:10.1017/S000711452200037X

Maternal folic acid supplement use/dietary folate intake from preconception to early pregnancy and neurodevelopment in 2-year-old offspring: the Japan Environment and Children's Study

Taeko Suzuki[1,2,3], Toshie Nishigori[2,4], Taku Obara[5], Toshio Masumoto[6], Miyuki Mori[1,2,7], Tsuyoshi Murata[2,8], Hyo Kyozuka[2,8], Yuka Ogata[2], Akiko Sato[2], Mari Sampei[2,3], Toshifumi Takahashi[2,9], Kosei Shinoki[2], Mitsuaki Hosoya[2,4], Keiya Fujimori[2,8], Seiji Yasumura[2,10], Koichi Hashimoto[2,4], Aya Goto[2,11] and Hidekazu Nishigori[1,2]* The Japan Environment and Children's Study Group

[1]*Department of Development and Environmental Medicine, Fukushima Medical Center for Children and Women, Fukushima Medical University Graduate School of Medicine, 1 Hikarigaoka, Fukushima-city, Fukushima 960-1295, Japan*
[2]*Fukushima Regional Center for the Japan Environmental and Children's Study, Fukushima, Japan*
[3]*Preparing section for School of Midwifery, Fukushima Medical University, Fukushima, Japan*
[4]*Department of Pediatrics, Fukushima Medical University School of Medicine, Fukushima, Japan*
[5]*Department of Pharmaceutical Sciences, Tohoku University Hospital, Sendai, Japan*
[6]*Division of Health Administration and Promotion, Faculty of Medicine, Tottori University, Yonago, Japan*
[7]*Department of Midwifery and Maternal Nursing, Fukushima Medical University School of Nursing, Fukushima, Japan*
[8]*Department of Obstetrics and Gynecology, Fukushima Medical University School of Medicine, Fukushima, Japan*
[9]*Fukushima Medical Center for Children and Women, Fukushima Medical University, Fukushima, Japan*
[10]*Department of Public Health, Fukushima Medical University School of Medicine, Fukushima, Japan*
[11]*Center for Integrated Science and Humanities, Fukushima Medical University School of Medicine, Fukushima, Japan*

(Submitted 6 July 2021 – Final revision received 20 January 2022 – Accepted 31 January 2022 – First published online 4 February 2022)

Abstract

We evaluated the association between maternal prenatal folic acid supplementation/dietary folate intake and motor and cognitive development in 2-year-old offspring using data from the Japan Environment and Children's Study database. Neurodevelopment of 2-year-old offspring were evaluated using the Kyoto Scale of Psychological Development 2001. In total, data of 3839 offspring were analysed. For folic acid supplementation, a multiple regression analysis showed that offspring of mothers who started using folic acid supplements before conception had a significantly lower developmental quotient (DQ) in the postural-motor DQ area than offspring of mothers who did not use them at any time throughout their pregnancy (partial regression coefficient (B) −2·596, 95 % CI −4·738, −0·455). Regarding daily dietary folate intake from preconception to early pregnancy, a multiple regression analysis showed that the group with ≥ 200 μg had a significantly higher DQ in the language-social area than the group with <200 μg. The DQ was higher in the ≥ 400 μg group (B 2·532, 95 % CI 0·201, 4·863) than the 200 to <400 μg group (B 1·437, 95 % CI 0·215, 2·660). In conclusion, our study showed that maternal adequate dietary folate intake from preconception to early pregnancy has a beneficial association with verbal cognition development in 2-year-old offspring. On the other hand, mothers who started using folic acid supplements before conception had an inverse association with motor development in 2-year-old offspring. There were no details on the amount of folic acid in the supplements used and frequency of use. Therefore, further studies are required.

Key words: Folic acid: Folate: Neurodevelopment: Developmental quotient: Cognitive development

Folate is important for fetal neurodevelopment and is an essential cofactor in DNA and RNA synthesis, DNA methylation processes, among others[1–4]. Previous studies have established that preconception folic acid supplementation of mothers reduces the risk of neural tube defects[5,6]. Recent studies have also shown that preconception or early pregnancy folic acid supplement use and adequate folate intake from food may be beneficial for their offspring's brain development and function. However,

Abbreviations: DQ, developmental quotient; JECS, Japan Environment and Children's Study.

* **Corresponding author:** Hidekazu Nishigori, email nishigo@fmu.ac.jp

existing studies in humans have controversial findings[7–9]. Nevertheless, the effects of maternal prenatal folic acid supplement use and dietary folate intake on offspring's neurodevelopment after birth have not been reported in Japan. In Japan, there is no mandatory folic acid food fortification policy, and the use of prenatal folic acid supplements is not as widespread as in other developed countries[10]. There are also differences resulting from race and genetic factors[11]. Therefore, Japanese research findings would be expected to provide new insights into prenatal folic acid supplement use/dietary folate intake and offspring neurodevelopment. To obtain evidence, we evaluated the association between maternal prenatal folic acid supplement use and dietary folate intake and motor and cognitive development in 2-year-old offspring using the Japan Environment and Children's Study (JECS) database.

Methods

Design and participants

JECS is a nationwide prospective birth cohort study involving 100 000 mother–offspring pairs, and the study started in 2011[12,13]. JECS is ongoing and was planned to continue until the children turn 13 years of age. Trained examiners evaluated the motor and cognitive development of approximately 5000 offspring selected as a Sub-Cohort Study of the JECS[14]. The dataset of 2-year-old offspring's test results were provided in 2020.

Ethical approval

The JECS protocol has been published elsewhere[12,13]. This study was conducted according to the guidelines laid down in the Declaration of Helsinki. It was reviewed and approved by the Ministry of the Environment's Institutional Review Board on Epidemiological Studies (no. 100910001) and by the ethics committees of all participating institutions. Written informed consent was obtained from all participants. From the JECS cohort, a Sub-Cohort Study comprising 5 % of the participating offspring, who were randomly selected and met the eligibility criteria, was extracted[14]. Extended outcome measurements of the Sub-Cohort Study were planned, including face-to-face interviews by trained personnel to evaluate neurological development using the Kyoto Scale of Psychological Development 2001 (KSPD) for 2-year- and 4-year-old offspring[14]. The present study used the jecs-ta-20190930 dataset, which was revised in April 2020. The dataset contains neurological developmental results of 2-year-old offspring by KSPD. Because this study was focused on offspring from singleton pregnancies, multiple birth offspring were excluded from the study.

Exposure: maternal folic acid supplement use

The Ministry of Health, Labor and Welfare in Japan recommended 400 μg/d of supplementary folic acid for pregnant women and women intending to get pregnant[15]. A face-to-face interview during pregnancy was conducted for pregnant women to assess folic acid supplementation and other supplementations[16,17]. In this study, the use of multivitamin supplements was not considered as folic acid supplements. This is because it was unknown whether all multivitamin supplements contained folic acid.

Participants were classified into four groups, based on the time of initiation of folic acid supplementation: (1) preconception users (started before conception), (2) early post-conception users (within 12 weeks of gestation), (3) late post-conception users (after 12 weeks of gestation) and (4) non-users (non-use of folic acid supplements before conception and during gestation).

Exposure: maternal dietary folate intake

A semi-quantitative FFQ was used to estimate dietary folate intake from foods[16]. The FFQ comprised the list of foods with standard portion sizes commonly consumed in Japan[18]. The validity of the FFQ for estimating dietary folate intake has been evaluated previously[18]. Participants reported the daily, weekly or monthly frequencies of food consumption over the previous year. The mother's FFQ was administered during the first and second trimester of gestation, at median 14·6 (interquartile range 12·0–18·0) weeks of gestation.

The FFQ is not designed to estimate folic acid[16,18]. In Japan, there is no mandatory folic acid food fortification policy. To the best of our knowledge, voluntary folic acid food fortification is not also common in Japan.

The Ministry of Health, Labor and Welfare in Japan recommends an estimated average requirement for total dietary folate, for example, from natural food sources, as follows: an intake of ≥ 200 μg/d dietary folate for adult women and ≥ 400 μg/d for pregnant women[15]. Therefore, the dietary folate intake of study participants was also classified into three groups (<200 μg, 200 μg to <400 μg and ≥ 400 μg).

Outcome: psychological development in 2-year-old offspring

The KSPD is a standardised developmental assessment tool for Japanese children covering the postural-motor (P-M), cognitive-adaptive (C-A) and language-social (L-S) areas[19,20]. The P-M, C-A and L-S areas correspond to the motor, non-verbal cognitive and verbal cognitive development. Scores from these three areas are combined to form the developmental quotient (DQ). The DQ was calculated by dividing the developmental age in days by the chronological age in days and multiplying the quotient by 100. For the reliability of administration, the interviewers were trained and certified by the JECS. Administrative procedures and evaluations were strictly standardised to ensure interviewers' reliability in this survey.

Statistical analysis and covariables

We compared mothers' characteristics and their offspring data on psychological development using ANOVA. Multiple regression analysis was used to assess the association between maternal prenatal folic acid intake/dietary folate intake and offspring psychological development. Multiple regression analyses were adjusted for maternal age at delivery, maternal BMI (kg/m^2) before pregnancy, infertility treatment, unexpected pregnancies, parity, marital status, maternal highest level of education,

https://doi.org/10.1017/S000711452200037X Published online by Cambridge University Press

British Journal of Nutrition

Table 1. Characteristics of the participants (Mean values and standard deviations; numbers and percentages)

		Overall (*n* 3839)		Folic acid supplement user (*n* 1616)		Folic acid supplement non-user (*n* 2223)		Reference for multiple regression analysis
		n	%	*n*	%	*n*	%	
Maternal age at delivery								
Mean		32·1		32·4		31·8		
SD		4·8		4·7		4·9		Continuous variable
	≤ 20	8	0·2	0	0·0	8	0·4	
	20–24	232	6·0	66	4·1	166	7·5	
	25–34	2350	61·2	1006	62·3	1344	60·5	
	≥ 35	1249	32·5	544	33·7	705	31·7	
Paternal age at delivery								
Mean		33·6		33·7		33·5		
SD		6·0		5·9		6·1		
	≤ 20	1	0·0	0	0·0	1	0·0	
	20–24	95	2·5	30	1·9	65	2·9	
	25–34	1114	29·0	470	29·1	644	29·0	
	≥ 35	878	22·9	370	22·9	508	22·9	
	No answer	1751	45·6	746	46·2	1005	45·2	
Maternal BMI (kg/m^2) before pregnancy								
Median		21·3		21·3		21·3		
IQR		3·3		3·4		3·2		
	<18·5	607	15·8	254	15·7	353	15·9	
	18·5≤–<25·0	2818	73·4	1182	73·1	1636	73·6	Ref
	≥ 25·0	414	10·8	180	11·1	234	10·5	
Infertility treatment	No	3578	93·2	1463	90·5	2115	95·1	Ref
	Yes	261	6·8	153	9·5	108	4·9	
Unexpected pregnancy	No	3556	92·6	1533	94·9	2023	91·0	Ref
	Yes	283	7·4	83	5·1	200	9·0	
Parity	Primipara	1553	40·5	782	48·4	771	34·7	Ref
	Multipara	2286	59·6	834	51·6	1452	65·3	
Marital status	Married, common-law marriage	3797	98·9	1597	98·8	2200	99·0	Ref
	Divorce	17	0·4	7	0·4	10	0·5	
	Other	25	0·7	12	0·7	13	0·6	
Maternal highest level of education	College, university	1719	44·8	810	50·1	909	40·9	
	Senior high school	2001	52·1	763	47·2	1238	55·7	Ref
	Junior high school	119	3·1	43	2·7	76	3·4	
Paternal highest level of education	College, university	1617	42·1	752	46·5	865	38·9	
	Senior high school	2021	52·6	800	49·5	1221	54·9	Ref
	Junior high school	201	5·2	64	4·0	137	6·2	
Maternal smoking during pregnancy	No	3724	97·0	1582	97·9	2142	96·4	Ref
	Yes	115	3·0	34	2·1	81	3·6	
Paternal smoking during pregnancy	No	2294	59·8	1037	64·2	1257	56·6	Ref
	Yes	1545	40·2	579	35·8	966	43·5	
Maternal alcohol consumption during pregnancy	No	3392	88·4	1437	88·9	1955	87·9	Ref
	Yes	447	11·6	179	11·1	268	12·1	
Annual household income (×10^3 yen/year) during pregnancy	<4000	1398	36·4	543	33·6	855	38·5	Ref
	4000≤–<6000	1311	34·2	554	34·3	757	34·1	

https://doi.org/10.1017/S000711452200037X Published online by Cambridge University Press

Table 1. (*Continued*)

		Overall (*n* 3839)		Folic acid supplement user (*n* 1616)		Folic acid supplement non-user (*n* 2223)		Reference for multiple regression analysis
		n	%	*n*	%	*n*	%	
	≥ 6000	1130	29·4	519	32·1	611	27·5	
Pregnancy complications	No	3197	83·3	1299	80·4	1898	85·4	Ref
	Yes	642	16·7	317	19·6	325	14·6	
Obstetric labour complication	No	2030	52·9	783	48·5	1247	56·1	Ref
	Yes	1809	47·1	833	51·6	976	43·9	
Mode of delivery	Vaginal	3177	82·8	1313	81·3	1864	83·9	Ref
	Cesarean	662	17·2	303	18·8	359	16·2	
Maternal neuropsychiatric disorders	No	3460	90·1	1442	89·2	2018	90·8	Ref
	Yes	379	9·9	174	10·8	205	9·2	
Maternal Kessler 6 psychological distress scale score ≥ 5 during pregnancy	No	2581	67·2	1072	66·3	1509	67·9	Ref
	Yes	1258	32·8	544	33·7	714	32·1	
Sex of offspring	Male	1942	50·6	821	50·8	1121	50·4	
	Female	1897	49·4	795	49·2	1102	49·6	
Birth weight of off spring (g)								
Mean		3058·4		3046·7		3059·9		Continuous variable
SD		397·3		406·6		392·5		
	0≤–<1500	3	0·1	2	0·1	1	0·0	
	1500≤–<2500	266	6·9	117	7·2	149	6·7	
	2500≤–<4000	3530	92·0	1479	91·5	2051	92·3	
	≥ 4000	40	1·0	18	1·1	22	1·0	
Gestation week of delivery								
Mean		39·0		39·0		39·0		
SD		1·4		1·4		1·3		
	22≤–<28	0	0·0	0	0·0	0	0·0	
	28≤–<34	16	0·4	8	0·5	8	0·4	
	34≤–<37	127	3·3	60	3·7	67	3·0	
	37≤–<42	3690	96·1	1545	95·6	2145	96·5	Ref
	≥ 42	6	0·2	3	0·2	3	0·1	
Breast-feeding at the age of 1 year and 6 months	Yes	1240	32·3	534	33·0	706	31·8	
	No	2599	67·7	1082	67·0	1517	68·2	Ref
Family structure	Extended family	737	19·2	287	17·8	450	20·2	
	Nuclear family	3102	80·8	1329	82·2	1773	79·8	Ref
Number of offspring's siblings	0	1529	39·8	785	48·6	744	33·5	
	1	1548	40·3	639	39·5	909	40·9	
	≥ 2	762	19·9	192	11·9	570	25·6	
Maternal job after delivery	No	2029	52·9	884	54·7	1145	51·5	Ref
	Yes	1810	47·2	732	45·3	1078	48·5	
Age at which the offspring started attending at day care centre	Not attend	1963	51·1	865	53·5	1098	49·4	Ref
	0≤–<1	877	22·8	332	20·5	545	24·5	
	≥ 1	999	26·0	419	25·9	580	26·1	
FFQ: maternal dietary intake								
Gestational weeks of answer								
Median		14·6						
IQR		12·0–18·0						

Table 1. (*Continued*)

		Overall (*n* 3839)		Folic acid supplement user (*n* 1616)		Folic acid supplement non-user (*n* 2223)		Reference for multiple regression analysis
		n	%	*n*	%	*n*	%	
Folate intake (μg/d)								
Median		251		250		251		
IQR		186–342		185–338		187–344		
	0≤–<200	1152	30·0	489	30·3	663	29·8	Ref
	200≤–<400	2083	54·3	884	54·7	1199	53·9	
	400≤–<1000	589	15·3	239	14·8	350	15·7	
	≥ 1000(maximum2956)	15	0·4	4	0·3	11	0·5	
		Median	IQR	Median	IQR	Median	IQR	
Total energy content (kJ/d)		7075·7	5823·8–8796·5	7046·4	5840·6–8673·0	7092·4	5811·3–8876·0	Continuous variable
Protein (g/d)		57·2	45·7–73·3	57·5	46·1–73·2	56·7	45·2–73·3	
Amino acids (g/d)		21·4	16·9–27·5	21·7	17·3–27·4	21·3	16·6–27·5	Continuous variable
n-3 unsaturated fatty acids (g/d)		1·76	1·28–2·35	1·73	1·29–2·32	1·78	1·27–2·37	Continuous variable
Fe (mg/d)		6·5	5·2–8·5	6·5	5·2–8·5	6·5	5·1–8·4	Continuous variable
Ca (mg/d)		453	319–637	465	334–647	444	311–631	Continuous variable
Zn (mg/d)		7·0	5·7–8·8	7·1	5·7–8·8	7·0	5·7–8·9	
Vitamin A (μg/d)		417	276–636	422	285–632	413	269–641	Continuous variable
Vitamin B_{12} (μg/d)		3·9	2·5–5·7	3·8	2·6–5·7	3·9	2·5–5·7	Continuous variable
Vitamin C (mg/d)		84	55–123	82	54–120	84	56–125	Continuous variable
Supplements or tablet		*n*	%	*n*	%	*n*	%	
Folic acid supplements	No use	2223	57·9					Ref
	Preconception use	329	8·6					
	Early post-conception use	999	26·0					
	Late post-conception use	288	7·5					
Multivitamin supplements	No	3700	96·4	1520	94·1	2180	98·1	Ref
	Yes	139	3·6	96	5·9	43	1·9	
Fe preparations	No	3781	98·5	1588	98·3	2193	98·7	Ref
	Yes	58	1·5	28	1·7	30	1·4	
Trace element	No	3601	93·8	1390	86·0	2211	99·5	Ref
	Yes	238	6·2	226	14·0	12	0·5	
Kyoto Scale of Psychological Development		Mean	SD	Mean	SD	Mean	SD	
Total DQ		93·3	18·0	92·6	17·5	93·9	18·3	
Postural-motor DQ		94·0	10·5	94·1	10·9	93·9	10·2	
Cognitive-adaptive DQ		95·5	12·6	95·3	12·8	95·6	12·5	
Language-social DQ		92·3	14·9	92·8	15·6	91·6	14·3	

IQR, interquartile range; DQ, developmental quotient.
Folic acid supplement user included (1) preconception users (started before conception), (2) early post-conception users (within 12 weeks of gestation) and (3) late post-conception users (after 12 weeks of gestation).
Folic acid supplement non-user: non-use of folic acid supplements before conception and during gestation.
The 6-item Kessler Psychological Distress Scale (K6; total point scores ranged from 0 to 24).

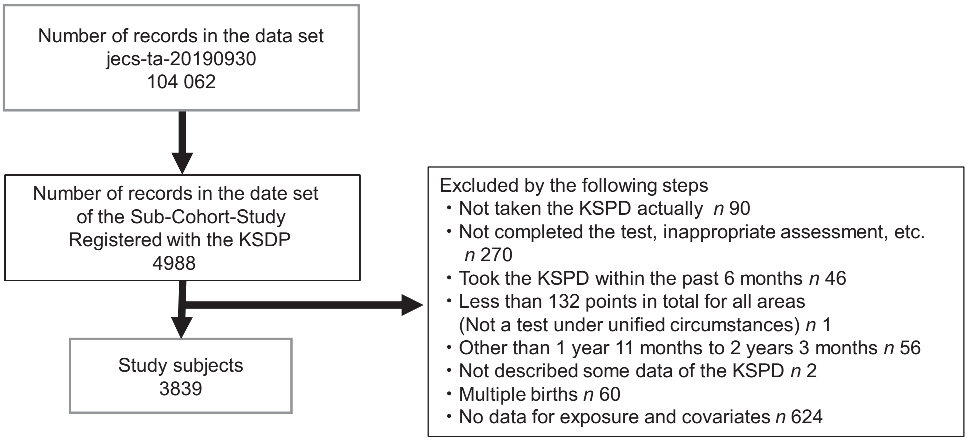

Fig. 1. Participant selection process flow chart. KSPD, Kyoto Scale of Psychological Development 2001.

Table 2. ANOVA for maternal folic acid supplement use and the Kyoto Scale of Psychological Development 2001 of 2-year-old offspring (Mean values and standard deviations, *n* 3839)

	Folic acid supplements	Mean	SD	*P*
Total DQ	No use	93·9	10·2	0·29
	Preconception use	93·4	11·5	
	Early post-conception use	94·5	10·6	
	Late post-conception use	93·7	10·9	
Postural-motor DQ	No use	93·9	18·3	0·01
	Preconception use	90·3	17·3	
	Early post-conception use	93·2	16·9	
	Late post-conception use	93·2	19·2	
Cognitive-adaptive DQ	No use	95·6	12·5	0·61
	Preconception use	94·8	12·7	
	Early post-conception use	95·6	12·7	
	Late post-conception use	94·9	13·0	
Language-social DQ	No use	91·9	14·3	0·13
	Preconception use	91·9	16·8	
	Early post-conception use	93·2	15·3	
	Late post-conception use	92·5	14·9	

DQ, developmental quotient.
Participants were classified into four groups based on folic acid supplementation start time: (1) preconception users (started before conception), (2) early post-conception users (within 12 weeks of gestation), (3) late post-conception users (after 12 weeks of gestation) and (4) non-users (non-use of folic acid supplements before conception and during gestation).

paternal highest level of education, maternal smoking status during pregnancy, paternal smoking status during pregnancy, maternal alcohol consumption during pregnancy, annual household income (×10^3 yen/year) during pregnancy, pregnancy complications, obstetric labour complications, mode of delivery, maternal neuropsychiatric disorders and maternal Kessler 6 (K6) psychological distress scale scores ≥ 5 during pregnancy[21–23]. Adjustments were also made for offspring's sex and birth weight, gestational week of delivery, breastfeeding at 1 year and 6 months postpartum, family structure, maternal job after delivery, day care centre attendance, multivitamin supplement use, Fe preparations, and trace element use. The dietary intake (FFQ) included energy content and nutrients, including amino acids, *n*-3 unsaturated fatty acids, Fe, Ca, vitamin A, vitamin B_{12} and vitamin C. There was no multicollinearity in this analysis. For reference, parity and number of children were found to be multicollinear. Total energy content, protein and Zn were found to be multicollinear.

All the analyses were performed using SAS statistical software, version 9.4 (SAS Institute Inc.).

Results

The records from 3839 offspring were analysed from 104 062 records in this dataset (Fig. 1). Table 1 shows the participants' characteristics, including 3839 single pregnancies and their offspring below 2 years of age. The maximum dietary folate intake of the ≥ 400 μg group was 2956 μg.

Folic acid supplements

The results of ANOVA for maternal folic acid supplement use and the KSPD of offspring is shown in Table 2. In the multiple regression analysis including dietary folate intake, a significantly lower postural-motor DQ score was observed in the preconception users' group (partial regression coefficient (B) −2·596, 95 % CI −4·738, −0·455, standardised partial regression coefficients (β) −0·040, $P = 0·02$) than in the non-users group (Table 3). In post-conception users, there was no significant association with any DQ score, compared with non-users.

Dietary folate intake

The results of ANOVA for maternal dietary folate intake and the KSPD of offspring is shown in Table 4. In the multiple regression analysis adjusted for folic acid supplement use, there was a significant higher score for language-social DQ in the 200 μg to <400 μg group (B 1·437, 95 % CI 0·215, 2·660, β 0·048, $P = 0·02$) and the ≥ 400 μg group (B 2·532, 95 % CI 0·201, 4·863, β 0·062, $P = 0·03$), compared with the <200 μg group (Table 5).

Discussion

Our study showed that preconception use of folic acid supplements was associated with lower motor development DQ in

Table 3. Multiple regression analysis for maternal folic acid supplement use and the Kyoto Scale of Psychological Development 2001 of 2-year-old offspring (Coefficient values and 95 % confidence intervals, *n* 3839)

	Folic acid suppliments use	Bivariate analysis				Multiple regression analysis							
						Adjust*				Adjusted for * and dietary folate intake			
		B	95 % CI	β	*P*	B	95 % CI	β	*P*	B	95 % CI	β	*P*
Total DQ	No use	ref				ref				ref			
	Preconception use	−0·492	−1·703, 0·720	−0·013	0·43	−0·615	−1·824, 0·593	−0·016	0·32	−0·599	−1·808, 0·610	−0·016	0·33
	Early post-conception use	0·603	−0·178, 1·384	0·025	0·13	0·633	−0·170, 1·435	0·027	0·12	0·645	−0·157, 1·448	0·027	0·12
	Late post-conception use	−0·162	−1·446, 1·122	−0·004	0·80	0·101	−1·144, 1·347	0·003	0·87	0·102	−1·143, 1·347	0·003	0·87
Postural-motor DQ	No use	ref				ref				ref			
	Preconception use	−3·576	−5·653, −1·499	−0·056	0·001	−2·609	−4·750, −0·467	−0·041	0·02	−2·596	−4·738, −0·455	−0·040	0·02
	Early post-conception use	−0·730	−2·069, 0·610	−0·018	0·29	−0·058	−1·480, 1·363	−0·001	0·94	−0·030	−1·451, 1·392	−0·001	0·97
	Late post-conception use	−0·685	−2·888, 1·517	−0·010	0·54	0·042	−2·164, 2·249	0·001	0·97	0·052	−2·154, 2·259	0·001	0·96
Cognitive-adaptive DQ	No use	ref				ref				ref			
	Preconception use	−0·769	−2·230, 0·692	−0·017	0·30	−0·664	−2·134, 0·807	−0·015	0·38	−0·652	−2·123, 0·819	−0·014	0·38
	Early post-conception use	0·086	−0·855, 1·028	0·003	0·86	0·182	−0·794, 1·157	0·006	0·72	0·187	−0·790, 1·163	0·007	0·71
	Late post-conception use	−0·642	−2·191, 0·906	−0·013	0·42	−0·237	−1·752, 1·278	−0·005	0·76	−0·238	−1·753, 1·277	−0·005	0·76
Language-social DQ	No use	ref				ref				ref			
	Preconception use	0·021	−1·699, 1·742	0·000	0·98	−0·646	−2·359, 1·066	−0·012	0·46	−0·609	−2·321, 1·102	−0·011	0·49
	Early post-conception use	1·318	0·209, 2·428	0·039	0·02	1·107	−0·029, 2·244	0·033	0·06	1·136	−0·001, 2·272	0·034	0·0502
	Late post-conception use	0·657	−1·167, 2·481	0·012	0·48	0·721	−1·043, 2·486	0·013	0·42	0·723	−1·041, 2·486	0·013	0·42

DQ, developmental quotient; B, partial regresion coefficient; β, standardised partial regression coefficients.
Participants were classified into four groups based on folic acid supplementation start time: (1) preconception use (started before conception), (2) early post-conception use (within 12 weeks of gestation), (3) late post-conception user (after 12 weeks of gestation) and (4) non-users (non-use of folic acid supplements before conception and during gestation).
* Adjusted for maternal age at delivery, maternal BMI (kg/m2) before pregnancy, infertility treatment, unexpected pregnancies, parity, marital status, maternal highest level of education, paternal highest level of education, maternal smoking status during pregnancy, paternal smoking status during pregnancy, maternal alcohol consumption during pregnancy, annual household income during pregnancy, pregnancy complications, obstetric labour complications, mode of delivery, maternal neuropsychiatric disorders, maternal Kessler 6 (K6) psychological distress scale scores ≥ 5 during pregnancy, offspring's sex and birth weight, gestation week of delivery, breast feeding at postpartum 1 years 6 month, family structure, maternal job after delivery, day care centre attendance, multivitamin supplement use, Fe preparations, trace element use, and the dietary intake (FFQ) included energy content and nutrients, including amino acids, *n*-3 unsaturated fatty acids, Fe, Ca, vitamin A, vitamin B_{12} and vitamin C.

https://doi.org/10.1017/S000711452200037X Published online by Cambridge University Press

Table 4. ANOVA for maternal folate intake from food and the Kyoto Scale of Psychological Development 2001 of 2-year-old offspring (Mean values and standard deviations, *n* 3839)

	Folate (µg) diet per d	Mean	SD	*P*
Total DQ	0≤–<200	93·6	10·5	0·23
	200≤–<400	94·2	10·4	
	≥ 400	94·3	10·7	
Postural-motor DQ	0≤–<200	93·5	18·0	0·62
	200≤–<400	93·1	17·8	
	≥ 400	93·8	18·4	
Cognitive-adaptive DQ	0≤–<200	95·0	12·5	0·39
	200≤–<400	95·7	12·6	
	≥ 400	95·6	12·8	
Language-social DQ	0≤–<200	91·3	15·2	0·03
	200≤–<400	92·6	14·6	
	≥ 400	92·9	15·1	

DQ, developmental quotient.

2-year-olds. In contrast, it was not associated with non-verbal cognitive or verbal cognitive development. Post-conception use of folic acid supplementation was not associated with motor, non-verbal cognitive or verbal cognitive development. Regarding dietary folate intake, adequate folate intake from preconception to early pregnancy was associated with higher DQ of verbal cognitive developments in 2-year-old offspring. However, dietary folate intake was not associated with non-verbal cognitive development or motor development.

Regarding the benefits of folic acid/folate, animal studies have shown that its deficiency or excessive use affects neurodevelopment in offspring[9]. However, from previous studies in humans, maternal folic acid supplement use/dietary folate intake and neurodevelopment in offspring is inconclusive[9]. Since our study involved 2-year-olds, we referred to previous studies in offspring up to 3 years old[9]. In a Spanish cohort study, offspring of mothers whose folic acid intake was less than 400 µg/d or 1000–5000 µg/d in early pregnancy had higher mental development scores than offspring of mothers who received 400 µg/d of folic acid, assessed with Bayley Scales of Infant and Toddler Development (BSID)-I in 1-year-old offspring[24]. However, folic acid intakes above 5000 µg/d had lower motor scores[24]. In the Greece cohort study, maternal supplemental folic acid intake of 5000 µg/d in early pregnancy was associated with higher receptive and expressive communication scores, assessed with BSID-III in 18-month-old offspring[25]. Nevertheless, folic acid intake exceeding 5000 µg/d was not associated with any of the BSID-III domains[25]. In the US cohort study, maternal folate intake from food and supplements of 600 µg/d in early pregnancy was associated with increased receptive language scores, assessed with Peabody Picture Vocabulary Test III in 3-year-old offspring. This association was even stronger for 600 µg/d of folic acid from supplements[26]. Nevertheless, no association was found between folate intake during the first and second trimester of pregnancy and fine motor or visual abilities, assessed with the Wide Range Assessment of Visual Motor Abilities test in 3-year-old offspring[26]. The following studies did not provide information on the amount of folic acid supplements; however, these has been included for reference. In a Polish cohort study, maternal use of folic acid supplements

Table 5. Multiple regression analysis for maternal folate intake from food and the Kyoto Scale of Psychological Development 2001 of 2-year-old offspring (Coefficient values and 95 % confidence intervals, *n* 3839)

		Bivariate analysis				Multiple regression analysis				Multiple regression analysis			
						Adjust*				Adjusted for * and folic acid suppliment use			
	Folate (µg) diet per d	*B*	95 % CI	*β*	*P*	*B*	95 % CI	*β*	*P*	*B*	95 % CI	*β*	*P*
Total DQ	0≤–<200	ref				ref				ref			
	200≤–<400	0·608	−0·145, 1·361	0·029	0·11	0·633	−0·230, 1·496	0·030	0·15	0·633	−0·230, 1·496	0·030	0·15
	≥ 400	0·685	−0·345, 1·715	0·024	0·19	1·095	−0·551, 2·740	0·038	0·19	1·120	−0·526, 2·766	0·039	0·18
Postural-motor DQ	0≤–<200	ref				ref				ref			
	200≤–<400	−0·431	−1·724, 0·861	−0·012	0·51	0·149	−1·381, 1·679	0·004	0·85	0·122	−1·408, 1·651	0·003	0·88
	≥ 400	0·303	−1·466, 2·072	0·006	0·74	1·909	−1·007, 4·825	0·039	0·20	1·875	−1·041, 4·792	0·038	0·21
Cognitive-adaptive DQ	0≤–<200	ref				ref				ref			
	200≤-<400	0·622	−0·286, 1·530	0·025	0·18	0·512	−0·538, 1·562	0·020	0·34	0·508	−0·542, 1·558	0·020	0·34
	≥ 400	0·539	−0·703, 1·781	0·016	0·39	0·600	−1·402, 2·601	0·017	0·56	0·602	−1·401, 2·605	0·017	0·56
Language-social DQ	0≤–<200	ref				ref				ref			
	200≤–<400	1·312	0·242, 2·381	0·044	0·02	1·434	0·212, 2·657	0·048	0·02	1·437	0·215, 2·660	0·048	0·02
	≥ 400	1·580	0·117, 3·043	0·039	0·03	2·484	0·153, 4·815	0·061	0·04	2·532	0·201, 4·863	0·062	0·03

DQ, developmental quotient; B, partial regression coefficient; β, standardised partial regression coefficients.

* Adjusted for maternal age at delivery, maternal BMI (kg/m^2) before pregnancy, infertility treatment, unexpected pregnancies, parity, marital status, maternal highest level of education, paternal highest level of education, maternal smoking status during pregnancy, paternal smoking status during pregnancy, maternal alcohol consumption during pregnancy, annual household income during pregnancy, pregnancy complications, obstetric labour complications, mode of delivery, maternal neuropsychiatric disorders, maternal Kessler 6 (K6) psychological distress scale scores ≥ 5 during pregnancy,offspring's sex and birth weight, gestation week of delivery, breast feeding at postpartum 1 year 6 months, family structure, maternal job after delivery, day care centre attendance, multivitamin supplement use, Fe preparations, trace element use and the dietary intake (FFQ) included energy content and nutrients, including amino acids, *n*-3 unsaturated fatty acids, Fe, Ca, vitamin A, vitamin B_{12} vitamin C.

started during the periconception period was not associated with BSID-III scores in 2-year-old offspring[27]. In a Norwegian cohort study, maternal use of folic acid supplements from the eighth week of pregnancy was associated with a reduced risk of severe language delay, as reported by parents using the language grammar scale of the Age-Specific Questionnaire (ASQ) in 3-year-old offspring[28]. However, there was no association between maternal folic acid supplementation before the eighth week of pregnancy and offspring's gross motor skills, assessed with ASQ in 3-year-old offspring[28]. In the US cohort study, maternal folic acid use was associated with a decreased risk of gross motor skill delay, assessed with the Denver screening test in 3-year-old offspring[29]. However, there was no association between the use of periconceptional folic acid supplements and language or fine motor development[29].

Compared with the findings of the aforementioned studies, our study showed that the use of folic acid supplements before pregnancy was associated with significantly lower motor skills in 2-year-old offspring, when compared with the non-use group. Although previous studies have controversial findings, a similar study showed that maternal folic acid intake of more than 5000 µg/d was associated with lower motor scores in a Spanish cohort study[29]. In our JECS survey, there was no information on the amount of folic acid in the supplements. Therefore, the association between folic supplement use and neurodevelopment in offspring needs to be examined in more detail, including its time of initiation and the amount of folic acid in the supplements. For reference, in Japan, folic acid supplements are available products from various companies. In general, it is recommended that pregnant women or planning to become pregnant be supplemented with 400 µg/d of folic acid, not exceeding 1000 µg/d[15].

Regarding dietary folate intake, our study showed that the 2-year-old offspring of mothers who took ≥ 200 µg daily dietary folate from preconception to early pregnancy had a higher DQ of verbal cognitive developments than the offspring of those who took <200 µg. The DQ was even higher in the ≥ 400 µg group (up to 2956 µg) than the 200 µg to <400 µg group. The Ministry of Health, Labor and Welfare in Japan recommends a daily intake of ≥ 200 µg of dietary folate for adult women and ≥ 400 µg/d for pregnant women[15]. Our study showed a beneficial association between dietary folate intake and verbal cognition development of 2-year-old offspring. This was similar to the findings of the aforementioned cohort studies in Greece, the USA and Norway[25,26,28]. These findings suggest that, clinically, maternal adequate dietary folate intake from preconception to early pregnancy is associated with better cognitive development of the offspring.

This study had limitations: first, the lack of detailed information on folic acid supplement use and the fact that dietary folate intake evaluation was self-reported by the FFQ. Second, the retrospective collection of information for maternal supplement usage, which in the case of the preconceptional period, was at least about 3–4 months before the interviews; therefore, the recollection may not be very accurate. However, this was an objective investigation in which trained interviewers assessed offspring's neurodevelopment; this was the study's strength.

In conclusion, our study showed that maternal adequate dietary folate intake from preconception to early pregnancy has a beneficial association with verbal cognition development in 2-year-old offspring. On the other hand, mothers who started using folic acid supplements before conception had an inverse association with motor development in 2-year-old offspring. There were no details on the amount of folic acid in the supplements used and frequency of use. Therefore, further studies are required.

Acknowledgements

The authors are grateful to all the participants of the study. Members of the JECS group as of 2021 include Michihiro Kamijima (principal investigator, Nagoya City University, Nagoya, Japan), Shin Yamazaki (National Institute for Environmental Studies, Tsukuba, Japan), Yukihiro Ohya (National Center for Child Health and Development, Tokyo, Japan), Reiko Kishi (Hokkaido University, Sapporo, Japan), Nobuo Yaegashi (Tohoku University, Sendai, Japan), Koichi Hashimoto (Fukushima Medical University, Fukushima, Japan), Chisato Mori (Chiba University, Chiba, Japan), Shuichi Ito (Yokohama City University, Yokohama, Japan), Zentaro Yamagata (University of Yamanashi, Chuo, Japan), Hidekuni Inadera (University of Toyama, Toyama, Japan), Takeo Nakayama (Kyoto University, Kyoto, Japan), Hiroyasu Iso (Osaka University, Suita, Japan), Masayuki Shima (Hyogo College of Medicine, Nishinomiya, Japan), Youichi Kurozawa (Tottori University, Yonago, Japan), Narufumi Suganuma (Kochi University, Nankoku, Japan), Koichi Kusuhara (University of Occupational and Environmental Health, Kitakyushu, Japan) and Takahiko Katoh (Kumamoto University, Kumamoto, Japan).

This study was funded by the Ministry of the Environment, Japan. The findings and conclusions of this article are solely the responsibility of the authors and do not represent the official views of the above government.

The authors' contributions are as follows: T. S., T. N., T. O., T. M, S. Y., K. H., A. G. and H. N. designed the study. T. O., H. K., Y. O., A. S., M. H., K. F., S. Y., K. H. and H. N. carried out the study. T. S., T. N. and H. N. analysed the data. T. S., T. N., T. O., T. M., M. M., T. M., H. K., Y. O., A. S., A. S., M. S., T. T., S. K., M. H., K. F., S. Y., K. H., A. G. and H. N. interpreted the findings. T. S., T. N. and H. N. wrote the paper.

There are no conflicts of interest.

References

1. Craciunescu CN, Brown EC, Mar MH, *et al.* (2004) Folic acid deficiency during late gestation decreases progenitor cell proliferation and increases apoptosis in fetal mouse brain. *J Nutr* **134**, 162–166.
2. Steegers-Theunissen RP, Obermann-Borst SA, Kremer D, *et al.* (2009) Periconceptional maternal folic acid use of 400 microg per day is related to increased methylation of the IGF2 gene in the very young child. *PLoS One* **4**, e7845.

3. Jadavji NM, Deng L, Malysheva O, *et al.* (2015) MTHFR deficiency or reduced intake of folate or choline in pregnant mice results in impaired short-term memory and increased apoptosis in the hippocampus of wild-type offspring. *Neurosci* **300**, 1–9.
4. Lee HS (2015) Impact of maternal diet on the epigenome during inutero life and the developmental programming of diseases in childhood and adulthood. *Nutrients* **7**, 9492–9507.
5. MRC Vitamin Study Research Group (1991) Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. *Lancet* **338**, 131–137.
6. Berry RJ, Li Z, Erickson JD, *et al.* (1999) Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. *N Engl J Med* **341**, 1485–1490.
7. Gao Y, Sheng C, Xie RH, *et al.* (2016) New perspective on impact of folic acid supplementation during pregnancy on neurodevelopment/autism in the offspring children – a systematic review. *PLOS ONE* **11**, e0165626.
8. Li M, Francis E, Hinkle SN, *et al.* (2019) Preconception and prenatal nutrition and neurodevelopmental disorders: a systematic review and meta-analysis. *Nutrients* **11**, 1628.
9. Naninck EFG, Stijger PC & Brouwer-Brolsma EM (2019) The importance of maternal folate status for brain development and function of offspring. *Adv Nutr* **10**, 502–519.
10. Ishikawa T, Obara T, Nishigori H, *et al.* (2020) Update on the prevalence and determinants of folic acid use in Japan evaluated with 91 538 pregnant women: the Japan Environment and Children's Study. *J Matern Fetal Neonatal Med* **33**, 427–436.
11. Nagasaki M, Yasuda J, Katsuoka F, *et al.* (2015) Rare variant discovery by deep whole-genome sequencing of 1070 Japanese individuals. *Nat Commun* **6**, 8018.
12. Kawamoto T, Nitta H, Murata K, *et al.* (2014) Rationale and study design of the Japan Environment and Children's Study (JECS). *BMC Public Health* **14**, 25.
13. Michikawa T, Nitta H, Nakayama SF, *et al.* (2018) Baseline profile of participants in the Japan Environment and Children's Study (JECS). *J Epidemiol* **28**, 99–104.
14. Sekiyama M, Yamazaki S, Michikawa T, *et al.* (2020). Study design and participants' profile in the Sub-Cohort Study in the Japan Environment and Children's Study (JECS). *J Epidemiol* (In the Press).
15. Ministry of Health, Labor and Welfare of Japan (2020) DRIs for Folate and Folic Acid. In Overview of Dietary Reference Intakes for Japanese, p. 232–237. https://www.mhlw.go.jp/content/10904750/000586553.pdf (accessed October 2021).
16. Iwai-Shimada M, Nakayama SF, Isobe T, *et al.* (2018) Questionnaire results on exposure characteristics of pregnant women participating in the Japan Environment and Children Study (JECS). *Environ Health Prev Med* **23**, 45.
17. Nishigori H, Obara T, Nishigori T, *et al.* (2017) Drug use before and during pregnancy in Japan: the Japan Environment and Children's Study. *Pharm* **5**, 21.
18. Yokoyama Y, Takachi R, Ishihara J, *et al.* (2016) Validity of short and long self-administered food frequency questionnaires in ranking dietary intake in middle-aged and elderly Japanese in the Japan Public Health Center-Based Prospective Study for the Next Generation (JPHC-NEXT) protocol area. *J Epidemiol* **26**, 420–432.
19. Society for the Kyoto Scale of Psychological Development Test (2008) *Shinpan K Shiki Hattatsu Kensahou 2001 Nenban (The Kyoto Scale of Psychological Development Test 2001)*. Kyoto, Japan: Nakanishiya Shuppan.
20. Koyama T, Osada H, Tsujii H, *et al.* (2009) Utility of the Kyoto Scale of Psychological development in cognitive assessment of children with pervasive developmental disorders. *Psychiatr Clin Neurosci* **63**, 241–243.
21. Kessler RC, Barker PR, Colpe LJ, *et al.* (2003) Screening for serious mental illness in the general population. *Arch Gen Psychiatr* **60**, 184–189.
22. Furukawa TA, Kawakami N, Saitoh M, *et al.* (2008) The performance of the Japanese version of the K6 and K10 in the World Mental Health Survey Japan. *Int J Meth Psychiatr Res* **17**, 152–158.
23. Sakurai K, Nishi A, Kondo K, *et al.* (2011) Screening performance of K6/K10 and other screening instruments for mood and anxiety disorders in Japan. *Psychiatr Clin Neurosci* **65**, 434–441.
24. Valera-Gran D, de la Hera MG, Navarrete-Muñoz EM, *et al.* (2014) Folic acid supplements during pregnancy and child psychomotor development after the first year of life. *JAMA Pediatr* **168**, e142611.
25. Chatzi L, Papadopoulou E, Koutra K, *et al.* (2012) Effect of high doses of folic acid supplementation in early pregnancy on child neurodevelopment at 18 months of age: the mother-child cohort 'Rhea' study in Crete, Greece. *Public Health Nutr* **15**, 1728–1736.
26. Villamor E, Rifas-Shiman SL, Gillman MW, *et al.* (2012) Maternal intake of methyl-donor nutrients and child cognition at 3 years of age. *Paediatr Perinat Epidemiol* **26**, 328–335.
27. Polańska K, Muszyński P, Sobala W, *et al.* (2015) Maternal lifestyle during pregnancy and child psychomotor development – Polish Mother and Child Cohort study. *Early Hum Dev* **91**, 317–325.
28. Roth C, Magnus P, Schjølberg S, *et al.* (2011) Folic acid supplements in pregnancy and severe language delay in children. *JAMA* **306**, 1566–1573.
29. Wehby GL & Murray JC (2008) The effects of prenatal use of folic acid and other dietary supplements on early child development. *Matern Child Health J* **12**, 180–187.