Association of antioxidant vitamins and oxidative stress levels in pregnancy with infant growth during the first year of life

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Abstract

Objective: Whereas there are numerous reports in the literature relating the impact of maternal nutritional status on subsequent birth outcome, much less is known about the long-term impact on infant growth after birth. Therefore, we conducted a prospective cohort study to investigate the association of maternal micronutrient status (vitamins A, C and E, folate) and oxidative stress status in pregnancy with infant growth during the first year of life.

Design: Prospective cohort study.

Setting: Outpatient clinic of obstetrics, Ewha Womans University Hospital, Seoul, South Korea.

Subjects and methods: Two groups were constructed for this study – the Ewha pregnancy cohort ($n = 677$) and the infant growth cohort comprising follow-up live newborns of all the recruited pregnant women ($n = 317$). Maternal serum vitamin and urinary oxidative stress levels were collected and infant weights and heights were measured at birth and at 6 and 12 months after birth.

Results: Division of the subjects into folate-deficient and normal groups revealed that infant weight and height at 0, 6 and 12 months were adversely affected by folate deficiency. High maternal vitamin C was associated with increased infant weight and height at birth and after birth.

Conclusion: Our findings indicate the importance of preventing folate deficiency and supplementing vitamin C during pregnancy.

Maternal nutrition in pregnancy can have a profound and sustained impact on the health, development and well-being of the foetus, newborn and infant, as well as on the pregnant woman herself. The foetus is totally dependent on the mother for the supply of nutrients essential to maintain its life. An optimal maternal nutritional status has been shown to reduce the risks of a preterm birth and low birth weight[1–3], improve postnatal growth[4,5], and reduce both maternal and infant morbidity and mortality[6–9]. Despite a trend of improved infant growth in most countries due to enhanced intakes of macronutrients[10], a low maternal nutritional status during the critical stage of foetal development subsequently contributes to growth retardation in the offspring and adverse health outcomes in later life[11]. In particular, macronutrients and associated foetal growth have been widely emphasised, but deficiencies in maternal micronutrients and induced oxidative stress have not received much attention. Therefore, adverse foetal environments caused by maternal micronutrient deficiency or a high level of oxidative stress need to be investigated from a public health perspective, with identification of their pivotal role in infant growth.

Many observational studies, as well as clinical trials, have aimed at determining the association of maternal vitamin or mineral status with birth outcomes[12–18]. Vitamins A, C and E are essential in a wide range of physiological processes such as pregnancy, promotion of general growth, ameliorating increased oxidative stress during pregnancy, and the provision of antioxidant defence to the foetus[19,20]. Downie *et al.* found that maternal vitamin A supplementation resulted in increased foetal growth[21]. However, two large randomised trials in Nepal and Indonesia found that vitamin A had no effect on foetal growth or loss[22,23]. Several observational studies found associations between low vitamin C status and low birth weight[24–26], and another study found that improved maternal vitamin E and C status was associated with increased birth size[27]. Folate plays a significant role

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as a coenzyme in the synthesis of DNA, RNA and certain amino acids, and in formulating normal foetal development[28,29]. However, there is a large body of literature reporting inconsistent effects of folate on pregnancy: some studies have shown its beneficial effects on improving birth weight and decreasing preterm delivery[26,30,31], but others have found no such associations[32]. Maternal oxidative stress due to environmental factors such as smoke, air pollution and chemicals has been shown to counteract the actions of the above-mentioned antioxidant vitamins in protecting the foetus from damage caused by oxygen free radicals[33,34].

While there is considerable literature relating the impact of maternal nutritional status such as vitamins and minerals on subsequent birth outcomes such as birth weight and foetal development and growth, much less is known about the long-term impacts on infant growth after birth. According to the concept of foetal programming, adverse foetal environments might have a negative effect on postnatal development, and several studies have investigated the association between postnatal growth and maternal micronutrient status. However, these studies either found no association or were not conducted on a general population. For example, a randomised controlled trial on the effects of vitamin A on weight and height in children involved only anaemic children with low socio-economic status[35]. Another randomised double-blind trial found no association between vitamin A and infant growth at 1 year after birth[36]. Accordingly, further studies are needed to clarify the real situation. Therefore, the current study investigated the association of maternal micronutrient status (vitamins A, C and E, folate) and oxidative stress in pregnancy with infant growth during the first year of life.

Methods

Ewha pregnancy cohort

Two groups were constructed for this study: the Ewha pregnancy cohort and the infant growth cohort comprising follow-up live newborns of all the recruited pregnant women (Fig. 1). From August 2001 to September 2005, the Ewha pregnancy cohort was constructed from 835 healthy pregnant women who visited Ewha Womans University Hospital for prenatal care during gestational weeks 24–28 and agreed to participate in the study. Women in this population with twin births ($n = 28$) or with pregnancy-induced hypertension or gestational diabetes ($n = 130$) were excluded, and hence 677 subjects were examined. Anthropometric data, sociodemographic and behavioural information, and disease status were collected by trained nurses during visits by the women to the outpatient clinic of the university hospital. Gestational age of the neonate at delivery was assessed by the date of the last menstrual period and by ultrasound measurement.

Infant growth cohort

The infant group was constructed from infants who were born to mothers in the pregnancy cohort, and they were followed up during the first year of life. Among 811 infants born, 317 infants with follow-up data from birth to 1 year were analysed. Trained nurses working in delivery rooms collected information on birth outcomes based on medical records.

Maternal serum and urinary measurements

In order to obtain serum micronutrient status (folate, vitamins A, C and E), fasting venous blood was drawn from the subjects between 24 and 28 weeks of gestation. Serum was separated by centrifugation within 4 h and stored at $-70°C$ until analysis. The amount of serum folate was measured by a radioimmunoassay kit (Diagnostic Products Corporation). Plasma levels of vitamin A (retinol), vitamin C (L-ascorbic acid) and vitamin E (α-tocopherol) were measured using high-performance liquid chromatography (HPLC) on a Shimadzu SCL-10A system, under the conditions listed in Table 1[37,38]. α-Tocopherol and retinol were measured by Bieri's method. Two hundred microlitres of plasma was combined with 1.0 ml of hexane, vortexed, and then centrifuged for 10 min at 2000***g***. The hexane was removed, the organic solvent layer was evaporated to dryness under nitrogen and reconstituted with 100 μl of mobile phase solution, and then 10 μl of the reconstituted solution was subjected to HPLC analysis using tocopherol acetate (50 μg/ml) as an internal standard. For vitamin C analysis, 100 μl of the plasma was mixed with 100 μl of 5 % metaphosphoric acid, vortexed, and then centrifuged for 10 min at 3000***g***. The supernatant was filtered by a 0·45-μm syringe filter, and 10 μl of the sample was subjected to HPLC analysis.

During the same period, oxidative stress status was assessed by measuring the spontaneous urinary concentrations of malondialdehyde (MDA) and 8-hydroxydeoxyguanosine (8-OHdG). MDA was measured by HPLC on adduct obtained with thiobarbituric acid, and 8-OHdG was measured by competitive *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of oxidative DNA adducts.

Postnatal growth measurement

To assess the growth of infants during the first year of life, their weight and height were measured three times: at birth, and 6 and 12 months after birth. Birth weights and heights were routinely measured by trained nurses in the delivery room, and weights and heights after birth were measured during visits by the subjects to an outpatient clinic. In cases where measurement at the clinic was not possible, a questionnaire asking for information on weight, height and general development of the infant was mailed. The agreements of measured weight and height between the outpatient visit and mail were assessed using Spearman correlation analysis. The resulting very high
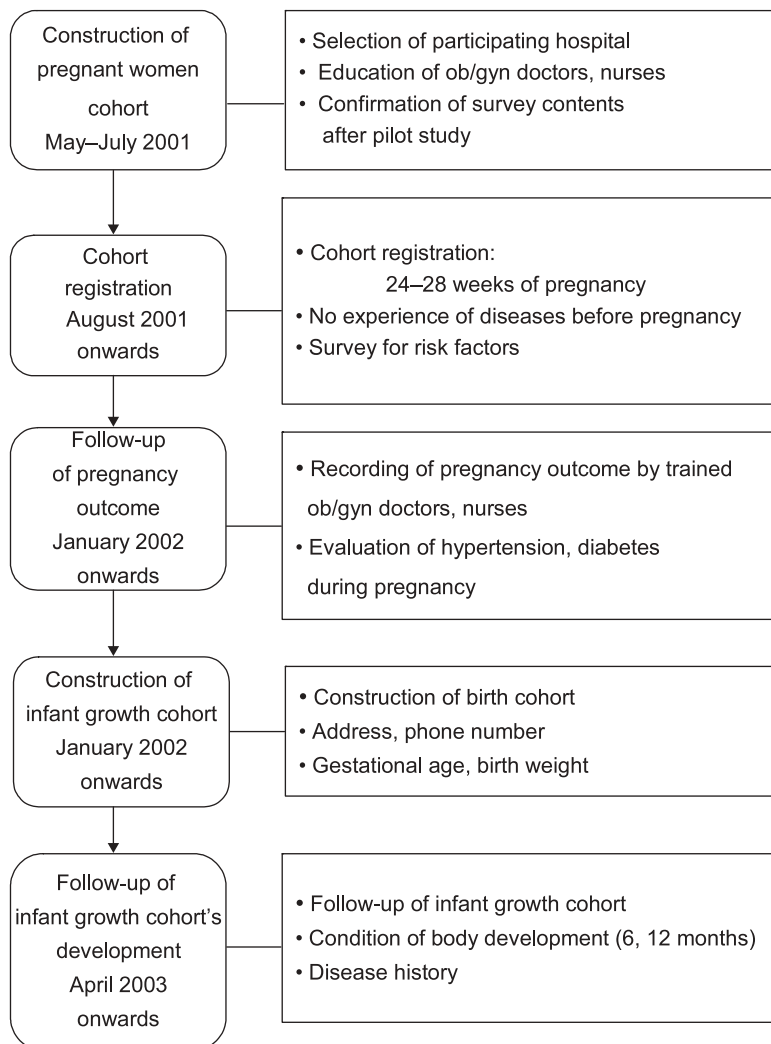
Fig. 1 Flowchart of cohort construction

Table 1 Conditions for high-performance liquid chromatography

Condition	L-Ascorbic acid	α-Tocopherol	Retinol
Column	Cap cell pack 18 (Shimadzu)	Cap cell pack 18 (Shimadzu)	Cap cell pack 18 (Shimadzu)
Mobile phase	0.01 M-KH_2PO_4 (PIC A, 10 ml/l)	Acetonitrile–methanol (75 : 25, v/v)	Acetonitrile–methanol (75 : 25, v/v)
Detector	UV, 254 nm	UV, 325 nm	UV, 292 nm
Flow rate	0.8 ml/min	1 ml/min	1 ml/min

UV, ultraviolet.

correlation ($r = 0.99$) indicated that mailed measurements could be used as a substitute for outpatient measurements. The study protocol was approved by the institutional review board of Ewha Womans University.

Statistical analysis

Owing to the absence of definite deficiency criteria for maternal vitamins excluding folate and oxidative stress, they were categorised into two groups (low/high) at the 25th, 50th or 75th percentile as determined by the distribution of serum or urinary concentrations among the entire cohort. An even distribution of possible confounders such as infant sex and gestational age between the two groups could prevent outcomes from being calculated due to the presence of infinite likelihood, and hence the cut-off points for vitamins A, C and E, and oxidative stress were determined in a different way.

The folate concentration was categorised into a deficient group ($< 6.8 \, \mu mol/l$) and a normal group[39].

Vitamin A was dichotomised into high and low groups at the 25th percentile, and vitamin C and oxidative stress were dichotomised at the 75th percentiles. Vitamin E was divided at the 50th percentile. The associations of maternal serum vitamin and oxidative stress levels with infant weight and height at 0, 6 and 12 months were assessed by repeated-measures analysis of variance using the MIXED procedure in the Statistical Analysis Systems statistical software package version 8·0 (SAS Institute, Cary, NC, USA). Possible confounders were dealt with using three adjustments: for infant sex (Model 1); for infant sex and gestational age (Model 2); and for infant sex, gestational age, breast milk, mother's age, weight, height and job, father's weight and height, and the person who cares for the infant (Model 3).

Results

Table 2 lists the number of infants followed at birth, 6 months and 12 months, and their mean weight and height. Median value of mother's age was 31 years, and median maternal weight and height were 54 kg and 160 cm respectively. About 70 % of the mothers had jobs; of the infants, 87 % were cared for by the mother and 76 % were breast-fed (Table 3). Most infants were exposed to second-hand smoke (83·0 %) and took nutrition supplements (66·5 %). The majority of infants were cared for at home (97·3 %), had experiences for visiting hospital (87·3 %) and did not have experiences for hospital admission (80·6 %). Nearly all fathers of infants had jobs.

Table 4 presents the infants' mean weights according to maternal vitamin and oxidative stress status while adjusting for infant sex, gestational age and the variables in Table 3 (breast milk, mother's age, weight and job, father's weight, person who cares for the infant). Infant weight at birth, 6 months and 12 months was lower in the folate-deficient group, with the differences being statistically significant ($P = 0.02$) after adjusting for infant sex (Model 1) and for infant sex and gestational age (Model 2). The high-vitamin C group exhibited elevated weight at birth, 6 months and 12 months with statistical significances, although the adjustments for infant sex (Model 1) and for infant sex and gestational age (Model 2) lowered the birth weight in this group slightly. The probability values for retinol in Models 1 and 2 were not calculated for the same reason of infinite likelihood as mentioned above.

The relationships between infant height at birth, 6 months and 12 months and maternal vitamin and oxidative stress status are indicated in Table 5. The low-folate group exhibited lower infant height at birth, 6 months and 12 months, with statistical significance ($P = 0.02$ and 0·03), after adjusting both for infant sex (Model 1) and for infant sex and gestational age (Model 2). The high-vitamin C group presented significantly higher infant

Table 2 Weight and height of infants at birth, 6 months and 12 months

	Birth			6 months			12 months		
	Mean	SD	n	Mean	SD	n	Mean	SD	n
Weight (g)	3190	528	804	8254	1087	385	10 008	1274	311
Height (cm)	49	3	794	69	4	277	77	4	268

SD, standard deviation.

Table 3 General characteristics of subjects ($n = 317$)

	Median (range) or n (%)
Mother's age (years)	31 (20, 47)
Person who cares for infant	
Mother	346 (86·5)
Grandparent	37 (9·3)
Relatives	4 (1·0)
Babysitter	10 (2·5)
Kindergarten	1 (0·3)
Others	2 (0·5)
Breast milk	
Yes	306 (75·6)
No	99 (24·4)
Mother's job	
No	299 (69·5)
Yes	131 (30·5)
Father's height (cm)	173 (155, 190)
Father's weight (kg)	71 (50, 110)
Mother's height (cm)	160 (142, 177)
Mother's weight (kg)	54 (32, 95)

height ($P = 0.01$) after adjusting for infant sex, gestational age, breast milk, mother's age, height and job, father's height and person who cares for the infant (Model 3). Similar to infant weight, the probability values for retinol in Models 1 and 2 were not calculated.

Discussion

To our knowledge, the current study is the first to reveal the association of maternal micronutrients and oxidative stress status with infant growth from birth to 1 year of life. Most of the previous studies investigating the associations of nutrients with infant growth have shown the impact of postpartum supplementation to mothers or infants. Stein et al. determined the impact of nutritional supplementation provided during the early childhood of women on the height of the subsequent generation[4]. Lind et al., Mwanri et al. and Schmidt et al. also reported the effects on growth of children[5,35,36]; however, they also administered nutritional supplements to the children or pregnant women participating in their studies.

The findings from this prospective cohort study of pregnant women and infants were similar to those from previous intervention studies. Similar to studies involving the administration of nutrient supplements, a high level of maternal serum nutrients was associated with increased

Table 4 Associations of maternal vitamin status at the 24–28th week of pregnancy with mean infant weight (Model 1 is adjusted for infant sex; Model 2 is adjusted for infant sex and gestational age; Model 3 is adjusted for infant sex, gestational age, breast milk, mother's age, mother's weight, father's weight, person who cares for the infant, mother's job)

| | Mean weight (g) | | | | | | | | | | | |
| | Model 1 | | | | Model 2 | | | | Model 3 | | | |
Maternal vitamin status	Birth	6 months	12 months	P	Birth	6 months	12 months	P	Birth	6 months	12 months	P
Folate (μmol/l)												
Low group ($n = 84$) ($<6\cdot8$*)	2970	7468	9784	0·02	2948	7451	9791	0·02	3018	7888	9943	0·52
High group ($n = 802$)	3051	8178	9846		3028	8149	9821		3050	8195	9928	
Vitamin A (μg/dl)												
Low group ($n = 221$) ($\leq72\cdot2$†)	3251	8173	10 057		3046	7955	9850		3085	7896	9843	0·71
High group ($n = 749$)	3274	8346	10 027		3065	8127	9815		3011	8099	9857	
Vitamin E (μg/dl)												
Low group ($n = 469$)	3070	8092	9823	0·91	3048	8063	9804	0·85	3020	8006	9857	0·12
High group ($n = 500$) ($\geq1939\cdot8$‡)	3068	8107	9832		3048	8086	9819		3138	8226	9964	
Vitamin C (μg/ml)												
Low group ($n = 687$)	3086	8057	9744	0·01	3060	8026	9726	0·004	3083	8000	9763	0·0003
High group ($n = 243$) ($\geq9\cdot0$§)	3053	8344	10 111		3039	8329	10 098		3119	8557	10 424	
MDA (μmol/g creatinine)												
High group ($n = 94$) ($\geq2\cdot9$§)	3184	7984	9697	0·08	3186	8001	9706	0·09	3245	7998	9755	0·42
Low group ($n = 308$)	3280	8250	9966		3275	8245	9966		3221	8351	9918	
8-OHdG (μg/g creatinine)												
High group ($n = 97$) ($\geq0\cdot1$§)	3246	8262	9725	0·60	3195	8202	9693	0·29	3138	8399	9649	0·54
Low group ($n = 298$)	3258	8162	10 006		3270	8178	10 021		3293	8277	10 051	

MDA, malondialdehyde; 8-OHdG, 8-hydroxydeoxyguanosine.
*Folate: cut-off according to Herbert and Das[39].
†Vitamin A (retinol): low group is lower than the 25th percentile.
‡Vitamin E (α-tocopherol): high group is higher than the 50th percentile.
§Vitamin C (L-ascorbic acid), MDA and 8-OHdG: high group is higher than the 75th percentile.

Table 5 Associations of maternal vitamin status at the 24–28th week of pregnancy with mean infant height (Model 1 is adjusted for infant sex; Model 2 is adjusted for infant sex and gestational age; Model 3 is adjusted for infant sex, gestational age, breast milk, mother's age, mother's height, father's height, person who cares for the infant, mother's job)

| | Mean height (cm) | | | | | | | | | | | |
| | Model 1 | | | | Model 2 | | | | Model 3 | | | |
Maternal vitamin status	Birth	6 months	12 months	P	Birth	6 months	12 months	P	Birth	6 months	12 months	P
Folate (μmol/l)												
Low group ($n = 84$) ($<6\cdot8$*)	48·3	67·6	76·1	0·02	48·2	67·3	76·0	0·03	49·8	70·5	77·5	0·61
High group ($n = 802$)	49·1	68·7	76·5		49·0	68·6	76·4		50·3	70·2	78·0	
Vitamin A (μg/dl)												
Low group ($n = 221$) ($\leq72\cdot2$†)	49·4	68·6	77·0		49·0	68·3	76·7		50·9	70·2	78·7	0·21
High group ($n = 749$)	49·3	68·9	76·5		49·0	68·5	76·2		50·4	70·2	77·8	
Vitamin E (μg/dl)												
Low group ($n = 469$)	49·1	68·8	76·4	0·30	49·0	68·7	76·3	0·32	50·3	70·2	77·9	0·33
High group ($n = 500$) ($\geq1939\cdot8$‡)	49·0	68·4	76·2		49·0	68·2	76·1		50·8	70·4	78·1	
Vitamin C (μg/ml)												
Low group ($n = 687$)	49·2	68·8	76·4	0·17	49·1	68·6	76·2	0·22	50·3	70·0	77·7	0·008
High group ($n = 243$) ($\geq9\cdot0$§)	49·4	69·2	76·9		49·2	69·0	76·7		51·0	70·8	78·7	
MDA (μmol/g creatinine)												
High group ($n = 94$) ($\geq2\cdot9$§)	48·8	68·8	74·4	0·11	48·9	69·1	74·5	0·15	49·4	69·2	75·2	0·98
Low group ($n = 308$)	49·3	68·7	76·3		49·2	68·6	76·3		48·9	68·7	76·1	
8-OHdG (μg/g creatinine)												
High group ($n = 97$) ($\geq0\cdot1$§)	48·9	69·0	75·6	0·64	48·7	68·6	75·4	0·19	48·3	69·9	75·4	0·55
Low group ($n = 298$)	49·2	68·8	76·3		49·3	68·9	76·3		49·4	69·0	76·4	

MDA, malondialdehyde; 8-OHdG, 8-hydroxydeoxyguanosine.
*Folate: cut-off according to Herbert and Das[39].
†Vitamin A (retinol): low group is lower than the 25th percentile.
‡Vitamin E (α-tocopherol): high group is higher than the 50th percentile.
§Vitamin C (L-ascorbic acid), MDA and 8-OHdG: high group is higher than the 75th percentile.

infant growth. After adjusting for already-known factors such as infant sex and gestational age (in Models 1 and 2), infant weight at birth, 6 months and 12 months was significantly lower in the folate-deficient group than in the normal group. However, when adjusting for already-known (infant sex and gestational age) and possible factors (breast milk, mother's age, weight and job, father's weight, person who cares for the infant) altogether (Model 3), statistical significance disappeared. This suggests that parents' characteristics play an important role in infant growth. Infant weight at birth and 6 and 12 months tended to be significantly higher in the high-vitamin C

group with the consideration of already-known and possible factors (Model 3); however, the effects at birth were unexpectedly opposite with adjustments for infant sex and gestational age (Models 1 and 2). The fact that all three adjustments for already-known or possible factors had statistical significance points out that vitamin C is a strong predictor for infant growth. Similar to weight, infant height at birth and after birth was significantly lower in the folate-deficient group after adjusting for already-known factors (infant sex and gestational age, Models 1 and 2). Also, statistical significance disappeared after adjusting for already-known and possible factors (Model 3) and height at 6 months was higher in the folate-deficient group. The high-vitamin C group was associated with significantly higher infant heights with the consideration of both already-known and possible factors (Model 3). A high level of oxidative stress (as assessed by the levels of MDA and 8-OHdG) tended to be associated with low infant weight and height, but this relationship was not statistically significant.

There have been few studies on the direct associations of maternal serum micronutrient levels with postnatal growth. Nevertheless, these associations can be indirectly postulated from the concept of foetal programming, which explains that poor intrauterine environments (such as maternal nutrition deficiency) may induce changes in biochemical mechanisms and consequently result in reduced postnatal growth and an increased likelihood of developing disease at childhood or adulthood. There are critical periods during which structural, physiological and metabolic developments occur *in utero*, and retardation at these times can cause enforced adaptation to undesirable conditions that result in a wide range of permanent disabilities[40]. Our study has also found that weight and height at birth and after birth were relatively low in the low-vitamin groups. In particular, folate deficiency was strongly associated with low birth and postnatal weight and height, and sufficient vitamin C was significantly associated with high birth and postnatal weight and height, indicating the importance of avoiding folate deficiency and supplementing vitamin C among the antioxidant vitamins. Although the effects exerted by vitamin A, vitamin E and oxidative stress were relatively similar to those of folate and vitamin C, there was no statistical significance. The protective effect of vitamin C on weight was shown only from 6 to 12 months; therefore, it is tempting to conclude that the level of vitamin C does not influence birth weight. However, this result is not definitive in our study sample, and hence further studies are needed.

Similar to micronutrients, there are no published reports concerning the association of maternal oxidative stress with postnatal growth, while there have been a few reports on its association with foetal growth. High oxidative stress was found to be related to adverse pregnancy outcomes including low birth weight[34,41],

and presumably this inverse association would persist into postnatal growth. Although there was no statistical significance, our study revealed a potential association between high oxidative stress during pregnancy and reduced infant weight and height both at birth and after birth. Future studies should employ larger samples to clarify the importance of reducing oxidative stress in pregnant women.

Several limitations of the present study merit discussion. First, due to a low follow-up rate, differing characteristics between infants who participated in the study and those who did not could cause selection bias. This study excluded non-participants who were likely to have lower maternal nutrition, higher oxidative stress, and lower birth weight and height, which would underestimate the association (towards the null). Second, the influence of socio-economic and environmental conditions surrounding the mother and the infant on the associations between maternal nutritional factors and infant growth should have been considered in detail. Direct or indirect indicators such as the mother's education level, the family's size and socio-economic status, and household conditions have been widely studied and identified as important factors in infant nutrition and growth[42]. Our study did collect information about the mother's and father's occupations, places to care for infants, breast-feeding, weaning food, supplementation, hospital visits and admission, frequent infectious diseases and passive smoking; however, due to considerable missing data, only a few factors such as breast milk, mother's age, job, weight and height, father's weight and height and person who cares for the infant were analysed and proved as important factors. An effective technique for reducing the non-reply rate should be established, and other important factors such as lifestyle patterns and socio-economic status should be examined.

Third, although the primary method of weight and height measurement was based on a standardised and precise assessment in the hospital, not all of the data could be obtained in this way. Data on infants who did not visit the hospital at 6 or 12 months were collected by a mailed questionnaire, and this might have generated measurement errors although our Spearman correlation analysis suggested that such errors would be small. Fourth, since there was no definite deficiency or excess criteria for vitamins A, C and E, and oxidative stress, we dichotomised each of these variables using the 25th, 50th or 75th percentile as the cut-off point so as to distribute possible confounders between low and high groups as unevenly as possible. Finally, this study was conducted at a single hospital, which limits its generalisability to all pregnant women.

The most noteworthy feature of this study is its prospective cohort design. In addition, the determination of maternal vitamin and oxidative stress status by means of serum and urine collected in midterm pregnancy served

to elucidate the temporal association. Moreover, a longitudinal data set was formed so as not to exclude infants who were not followed up by the end of study, which allowed infants having at least one postnatal weight and height measurement (at 6 or 12 months) to be included in the analysis.

This study has demonstrated that a deficiency of folate causes low infant weight and height and that a high maternal status of vitamin C generates high infant weight and height. These results indicate the importance of preventing folate deficiency and supplementing vitamin C during pregnancy. In addition, parental characteristics such as age, job, weight and height and breast-feeding play important roles in infant growth during the first year of life, necessitating strategies to focus on specific groups having these characteristics. We observed and followed up a relatively small number of infants only from birth to the first year of life at one hospital setting, which makes it is somewhat difficult to produce definitive conclusions. However, the present study provides good evidence that maternal antioxidant micronutrients influence infant growth during the first year after birth.

Acknowledgements

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