# Folic acid supplementation in pregnant women with hepatitis B surface antigen improves infant hepatitis B surface antibody mediated by infant IL-4

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## Abstract

Immunoprophylaxis has not completely eliminated hepatitis B virus (HBV) infection due to hyporesponsiveness to hepatitis B vaccine (HepB). We explored the impact of folic acid supplementation (FAS) in pregnant women with positive hepatitis B surface antigen (HBsAg) on their infant hepatitis B surface antibody (anti-HBs) and the mediation effect of infant interleukin-4 (IL-4). We recruited HBsAg-positive mothers and their neonates at baseline. Maternal FAS was obtained via a questionnaire, and neonatal anti-HBs and IL-4 were detected. Follow-up was performed at 11–13 months of age of infants, when anti-HBs and IL-4 were measured. We applied univariate and multivariate analyses. A mediation effect model was performed to explore the mediating role of IL-4. A total of 399 mother–neonate pairs were enrolled and 195 mother–infant pairs were eligible for this analysis. The infant anti-HBs geometric mean concentrations in the maternal FAS group were significnatly higher than those in the no-FAS group (383·8 mIU/ml, 95 % CI: 294·2 mIU/ml to 500·7 mIU/ml *v.* 217·0 mIU/ml, 95 % CI: 147·0 mIU/ml to 320·4 mIU/ml, z = −3·2, *P* = 0·001). Infants born to women who took folic acid (FA) within the first trimester were more likely to have high anti-HBs titres (adjusted *β*-value = 194·1, *P* = 0·003). The fold change in IL-4 from neonates to infants partially mediated the beneficial influence of maternal FAS on infant anti-HBs (24·7 % mediation effect) after adjusting for confounding factors. FAS during the first trimester to HBsAg-positive mothers could facilitate higher anti-HBs levels in infants aged 11–13 months partly by upregulating IL-4 in infants.

**Key words: Hepatitis B virus: Folic acid: IL-4: Immunisation: Mediation effect**

Hepatitis B vaccination is the primary means of preventing infections and unfavourable complications caused by hepatitis B virus (HBV). Nonetheless, the incidence of hepatitis B surface antibody (anti-HBs) below the protective level (< 10 mIU/ml) in infants aged 12 months is 2·6–15·9 % after hepatitis B vaccination[1,2]. The lack of protective anti-HBs levels and close contact with HBsAg-positive mothers put these infants at high risk for HBV infection. Infants are more likely to die of liver cirrhosis and advanced hepatocellular carcinoma in the future because of HBV infection during infancy[3].

Most existing studies have focused on HBV virological factors[4,5] and cytokines in infants[6] that cause hyporesponsiveness to hepatitis B vaccine (HepB) in these high-risk infants. However, the maternal nutrient supplementation, especially folic acid (FA), has been neglected. Although the latest Chinese guidelines recommend folic acid supplementation

(FAS) before and during pregnancy, the start and end times of these guidelines are inconsistent[7–9]. Moreover, no emphasis has been placed on how pregnant women with hepatitis B should take FA supplements. Although one team revealed that maternal FAS was positively associated with anti-HBs titres 7–8 months[10] and 5 years after hepatitis B vaccination in infants[11], the relationship between FAS in women with positive HBsAg and infant anti-HBs has not been reported. Furthermore, the possible causes of this phenomenon remain largely unknown.

Similar to the 'developmental origins of health and disease' theory[12], some scholars have noted that normal development of the fetal immunity as a potential advantageous intermediate outcome of adequate intrauterine folate might facilitate protective levels of immune response to vaccines after birth[13]. Previous work has shown that FAS is associated with an increase in levels of markers related to immunity, such as B lymphocyte response

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markers, innate immune proinflammatory pathways[14], antigen processing and presentation markers, nuclear factor-k-gene binding signaling pathways, tumor necrosis factor signalling[15], IL-8[16] and IL-4[17,18]. Previous research has also revealed a positive association between immune markers and HepB response, including T-cell receptor $\beta$-chain variable[19], NDRG1-expressing myeloid dendritic cell-2, CDKN1C-expressing myeloid dendritic cell-4[20], chemokine (C-X-C motif) ligand 12, IL-27[21], interferon-γ, IL-2[22], IL-17, IL-18[23] and IL-4[22,24,25]. Among the above immune markers, we noticed that IL-4 is related to both FAS and HepB response. FA, a coenzyme necessary for cell division and differentiation, is vital for fetal immune development[17]. FAS in pregnant mice contributed to thymus development, led to a normal number of differentiated immune cells being maintained in the offspring before birth[26] and upregulated IL-4 produced and secreted by immune cells[17]. As the survival of plasma cells and continuous production of antibodies are dependent on IL-4, IL-4 is an irreplaceable cytokine in the immune response including the HepB immune response[24,27]. This finding strongly suggests that IL-4 as a biomarker might mediate the conducive influence of maternal FAS on infant anti-HBs, which has not been reported. To what extent the causal relationship between maternal FAS and infant anti-HB might be explained by infant IL-4 remains unclear.

Infants of HBsAg-positive mothers are exposed to a high-risk biological environment for HBV for a long time before birth. These infants' immune statuses, such as IL-4 levels, may be weaker than those of healthy mothers, resulting in low antibody titres.

The research hypotheses are that FAS in pregnant women with HBsAg would increase infant anti-HBs and that infant IL-4 could play a mediating role between them. We therefore performed a cohort study to test the above hypothesis to provide suggestions for early nutritional intervention to minimise the incidence of low anti-HBs in these infants.

## Methods

### Participants and study procedures

A cohort study was conducted among HBsAg-positive pregnant women and their neonates at the Department of Obstetrics and Gynecology of the Third People's Hospital in Taiyuan from June 2011 to July 2013. All infants received three doses of recombinant HepB within 24 h after birth and at 1 and 6 months[28]. HBIG was administered to neonates within 24 h after birth. Follow-up was performed for infants aged 11 to 13 months. Women with hepatitis C virus, HIV or other viral infections were excluded. Twins were excluded. This study was conducted according to the guidelines presented in the Declaration of Helsinki. All procedures involving human subjects/patients were approved by the Ethics Committee of Shanxi Medical University. Written informed consent was obtained from all subjects.

### Ascertainment of folic acid supplementation

Mothers were interviewed face to face using the uniform, structured and detailed FAS questionnaires, which were completed by trained and qualified investigators. The FAS questionnaire included questions about FAS during 1 year prior to conception, in the first trimester (0–12 gestational weeks), the second trimester (13–27 gestational weeks) and the third trimester (28 gestational weeks until delivery). Women were asked at what stage of pregnancy they had started FAS, for how many weeks they had taken FA supplements, how many times a week they had taken FA supplements (frequency) and how many milligrams of FA supplements they took each time (dose).

### Demographic and clinical data collection

Baseline data included maternal age, education, gestational week and HBV infection status and neonatal sex, birth weight, birth length and Apgar score. Maternal age and education were obtained from in-person questionnaires with mothers after delivery. The gestational week and HBV infection status (trained experimenters), neonatal sex, birth weight (trained nurses), birth length (trained nurses) and Apgar score (qualified doctors) were from electrical medical records. Follow-up data including hepatitis B vaccination and feeding patterns were collected from each infant immunisation record and in-person questionnaires with mothers when their infants were 11–13 months old.

### Detection of infant IL-4 and hepatitis B surface antibody

Venous blood was collected from each neonate within 24 h after birth, before immunoprophylaxis and from every infant aged 11–13 months. Three millilitres of neonatal and infant femoral venous blood was collected and placed in a nonanti-coagulant tube. IL-4 was tested by MultiSciences Biotech Co., Ltd. using MAGPIX (Luminex) and ProcartaPlex Human IL-4 Simplex 96 tests (eBioscience). Anti-HBs were detected using an electrochemiluminescence immunoassay (Roche Diagnostics GmbH).

### Statistical analyses

We adopted the double-entry method to reduce information bias. Two trained data entry staff independently input the same questionnaire content into the Epidata 3·1. Subsequently, a consistency test was performed and the inconsistent data were rechecked. Statistical analyses were implemented with SAS version 9·4 software(Inc.). The missing data were deleted. Two-sided $P < 0.05$ was considered statistically significant. Descriptive statistics included mean ± SD, proportions or geometric mean concentrations (GMC) with 95 % CI as appropriate. The $\chi^2$ test, continuous corrected $\chi^2$ test, Fisher's exact test, separate variance estimation $t$ test or Mann–Whitney $U$ test were applied to compare the differences in characteristics between the two groups.

A linear regression model was applied. The dependent variable was infant anti-HB titres [continuous variable (mIU/ml)]. The independent variable was FAS during the first trimester (0 = no, 1 = yes). We reduced bias by adjusting for confounding factors. The adjusted variables were the maternal HBV DNA (0 = negative, 1 = positive), maternal HBeAg (0 = negative, 1 =

positive), nutrient supplementation during the 1 year prior to pregnancy (0 = no, 1 = yes), other nutrients supplementation during the first trimester (0 = no, 1 = yes), nutrients supplementation during the second trimester (0 = no, 1 = yes), nutrient supplementation during the third trimester, neonatal prematurity (0 = no, 1 = yes), neonatal anti-HB titres [continuous variable (mIU/ml)] and feeding patterns of infants (1 = breast feeding, 2 = artificial feeding, 3 = mixed feeding). Because all of the neonates had a birth weight ≥ 2500 g, we defined the birth weight as 0 = '2500–4000 g' and 1 = '> 4000 g'. 'Nutrient supplementation (yes) = 1' was that the pregnant woman had taken any of the nutrient supplements, and 'Nutrient supplementation (no) = 0' was that the woman had not taken any of the nutrient supplements. 'Other nutrient supplementation (yes) = 1' was that the pregnant woman had taken any nutrient supplements other than FA, and 'Other nutrient supplementation (no) = 0' was that the woman had not taken any nutrient supplements other than FA. Fold change of IL-4 from neonates to infants = (IL-4 level of infants aged 11–13 months) – (IL-4 level of neonates at birth). Afterwards, we adopted a mediation effect analysis using the Statistical Package for Social Science (SPSS) for Windows, Version 22.0 (SPSS Inc.) and PROCESS for SPSS 2·16·3. PROCESS does not provide $P$ values for the indirect effect; instead, significance of the indirect effect was assumed if the 95 % CI did not include 0. The mediation model included FA as an independent variable (X), the fold change of IL-4 from neonates to infants as a mediator (M) and infant anti-HBs as a dependent variable (Y). When the mediation analysis was conducted, we used bootstrapping to estimate the indirect effect. We used a linear regression model in the mediation analysis. For more information on the SPSS PROCESS, see http://www.processmacro.org[29].

### Sample size assessment

PASS 11·0·7 was applied to calculate the sample size. As the incidence of anti-HB below the protective level in infants aged 12 months was 2·6–15·9 % after hepatitis B vaccination[1,2], we used 2·6 % as the proportion that PASS 11·0·7 needed. The statistical power was 80 %, and the two-sided significance level was 0·05. The software output result was 163. To increase the study power, all eligible mother–infant pairs were included in the analysis.

### Results

#### Participant enrolment and follow-up

The subjects' selection flow diagram is depicted in Fig. 1. We enrolled 399 mother–neonate pairs and 195 mother–infant pairs who met these analysis requirements. The rate of loss to follow-up was 27·07 % (108/399). The key demographic, clinical and lab characteristics did not differ between the infants retained in the study and those lost to follow-up (online Supplementary Tables 1–3). The follow-up rate did not differ between the infants born to mothers with FAS and those born to mothers without FAS (online Supplementary Tables 4 and 5).
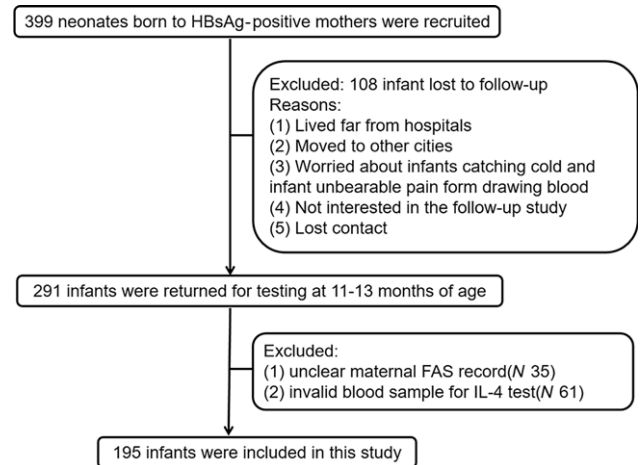


**Fig. 1.** Flow diagram of subjects' enrollment and follow-up for this study.

### Baseline and follow-up characteristics

Table 1 lists the baseline and follow-up characteristics by FAS or not during the first trimester. We found that all pregnant women took FA supplements seven times a week and 0·4 mg of FA each time. None of the 195 neonates were born with any malformations. The mean age (range) of the infants at follow-up was 12 (11–13) months. The percentage of HBV vaccine completion (3 doses) was 100 % in infants. The GMC (95 % CI) of anti-HBs in neonates was 2·13 (95 % CI: 2·04, 2·22) mIU/ml. The GMC (95 % CI) of infant anti-HB was 311·87 (95 % CI: 249·80, 389·38) mIU/ml. The neonatal anti-HBs GMC did not differ between the maternal FAS group and the no-FAS group [2·13, 95 % CI: 2·00, 2·27 *v.* 2·13, 95 % CI: 2·01, 2·25, z = 0·95, $P$ = 0·344]. The infant anti-HBs GMC in the maternal FAS group were significantly higher than those in the no-FAS group [383·82, 95 % CI: 294·24, 500·68 *v.* 217·04, 95 % CI: 147·04, 320·35, z = −3·23, $P$ = 0·001]. Infant IL-4 and fold change of IL-4 from neonates to infants were found to be significantly different between the maternal FAS group and the no-FAS group ($P$ < 0·001). Only one infant became infected with HBV (positive HBV DNA) in the maternal FAS group. Comparison of infant anti-HB in mothers with FAS and without FAS during different periods is shown in the Supplementary Table 6.

### The impact of folic acid supplementation during the first trimester on infant hepatitis B surface antibody

To explore prospective associations between maternal FAS during the first trimester and infant anti-HBs, we used the linear regression model listed in Table 2. The definition of the variable is displayed in Supplementary Table 7. FAS by HBsAg positive women was positively associated with high anti-HBs titres after hepatitis B vaccination (adjusted $\beta$-value = 194·12, $P$ = 0·003). Other variables were not associated with infant anti-HBs titres ($P$ > 0·05).

**Table 1.** HBsAg-positive mothers' and their neonates' baseline and follow-up characteristics
(Number and proportion and mean values and standard deviations)

| Characteristic | Number | Proportion | No Number | No Proportion | Yes Number | Yes Proportion | χ2/t/z | P |
|---|---|---|---|---|---|---|---|---|
| **Mothers** | | | | | | | | |
| Education | | | | | | | | |
|   High school and below high school | 104 | 53·33 | 45 | 63·38 | 59 | 47·58 | 4·92 | 0·086 |
|   Postsecondary specialised school | 47 | 24·10 | 12 | 16·90 | 35 | 28·23 | | |
|   University and above University | 44 | 22·56 | 14 | 19·72 | 30 | 24·19 | | |
| HBVDNA* | | | | | | | | |
|   − | 102 | 52·58 | 35 | 50·00 | 67 | 54·03 | 0·29 | 0·589 |
|   + | 92 | 47·42 | 35 | 50·00 | 57 | 45·97 | | |
| HBeAg | | | | | | | | |
|   − | 105 | 53·85 | 39 | 54·93 | 66 | 53·23 | 0·05 | 0·818 |
|   + | 90 | 46·15 | 32 | 45·07 | 58 | 46·77 | | |
| Gestational weeks (week) | | | | | | | | |
|   Mean | | 39·11 | | 39·05 | | 39·14 | −0·49 | 0·627 |
|   SD | | 1·11 | | 1·08 | | 1·14 | | |
| Age at delivery (year) | | | | | | | | |
|   Mean | | 27·70 | | 27·28 | | 27·94 | −1·13 | 0·258 |
|   SD | | 3·93 | | 4·28 | | 3·70 | | |
| Nutrient supplementation during 1 year prior to conception† | | | | | | | | |
|   No | 165 | 84·62 | 66 | 92·96 | 99 | 79·84 | 5·97 | 0·015 |
|   Yes | 30 | 15·38 | 5 | 7·04 | 25 | 20·16 | | |
| Other nutrient supplementation during the first trimester† | | | | | | | | |
|   No | 171 | 87·69 | 63 | 88·73 | 108 | 87·10 | 0·11 | 0·738 |
|   Yes | 24 | 12·31 | 8 | 11·27 | 16 | 12·90 | | |
| Nutrient supplementation during the second trimester† | | | | | | | | |
|   No | 102 | 52·31 | 52 | 73·24 | 50 | 40·32 | 19·61 | < 0·001 |
|   Yes | 93 | 47·69 | 19 | 26·76 | 74 | 59·68 | | |
| Nutrient supplementation during the third trimester† | | | | | | | | |
|   No | 150 | 76·92 | 60 | 84·51 | 90 | 72·58 | 3·62 | 0·058 |
|   Yes | 45 | 23·08 | 11 | 15·49 | 34 | 27·42 | | |
| **Neonates at birth** | | | | | | | | |
| Sex | | | | | | | | |
|   Female | 97 | 49·74 | 34 | 47·89 | 63 | 50·81 | 0·15 | 0·695 |
|   Male | 98 | 50·26 | 37 | 52·11 | 61 | 49·19 | | |
| Apgar score | | | | | | | | |
|   Mean | | 9·48 | | 9·40 | | 9·52 | −1·44 | 0·152 |
|   SD | | 0·56 | | 0·55 | | 0·56 | | |
| Birth weight (kg) | | | | | | | | |
|   Mean | | 3·41 | | 3·43 | | 3·39 | 0·58 | 0·564 |
|   SD | | 0·45 | | 4·07 | | 4·73 | | |
| Birth length (cm) | | | | | | | | |
|   Mean | | 50·06 | | 50·14 | | 50·01 | 0·59 | 0·555 |
|   SD | | 1·51 | | 1·56 | | 1·48 | | |
| IL-4 (pg/ml) | | | | | | | | |
|   Mean | | 9·99 | | 10·85 | | 9·48 | 1·67 | 0·097 |
|   SD | | 5·54 | | 5·19 | | 5·70 | | |
| GMC of anti-HB (mIU/ml) | | 2·13 | | 2·13 | | 2·13 | 0·95 | 0·344 |
|   95 % CI | | 2·04, 2·22 | | 2·01, 2·25 | | 2·00, 2·27 | | |
| **Infants aged 11 to 13 months** | | | | | | | | |
| Hepatitis B vaccination | | | | | | | | |
|   3 doses | 195 | 100 | 71 | 100 | 124 | 100 | − | − |
|   Less than 3 doses | 0 | 0 | 0 | 0 | 0 | 0 | | |
| Feeding patterns‡ | | | | | | | | |
|   Breast-feeding | 39 | 20·74 | 19 | 27·14 | 20 | 16·95 | 2·96 | 0·227 |
|   Artificial feeding | 117 | 62·23 | 39 | 55·71 | 78 | 66·10 | | |
|   Mixed feeding | 32 | 17·02 | 12 | 17·14 | 20 | 16·95 | | |
| IL-4 (pg/ml) | | | | | | | | |
|   Mean | | 11·57 | | 7·74 | | 13·76 | −5·50 | < 0·001 |
|   SD | | 7·88 | | 7·10 | | 7·49 | | |
| Fold change of IL-4 (pg/ml)§ | | | | | | | | |
|   Mean | | 1·58 | | −3·11 | | 4·26 | −5·23‖ | < 0·001 |
|   SD | | 10·79 | | 8·12 | | 11·10 | | |
| GMC of anti-HBs (mIU/ml) | | 311·87 | | 217·04 | | 383·82 | −3·23 | 0·001 |
|   95 % CI | | 249·80, 389·38 | | 147·04, 320·35 | | 294·24, 500·68 | | |

GMC, geometric mean concentrations.

\* This category excludes two mothers with insufficient samples for HBVDNA.

† 'Nutrient supplementation (yes) = 1' was that the pregnant woman had taken any of the nutrient supplements, and 'Nutrient supplementation (no) = 0' was that the woman had not taken any of the nutrient supplements. 'Other nutrient supplementation (yes) = 1' was that the pregnant woman had taken any nutrient supplements other than folic acid, and 'Other nutrient supplementation (no) = 0' was that the woman had not taken any nutrient supplements other than folic acid.

‡ This category excludes seven infants with missing data on feeding patterns.

§ Fold change of IL-4 = (IL-4 level of infants aged 11–13 months) − (IL-4 level of neonates at birth).

‖ We used separate variance estimation $t$ test due to heterogeneity of variance on fold change of IL-4 between the two groups.

**Table 2.** The impact of folic acid supplementation during the first trimester to hepatitis B surface antigen (HBsAg)-positive mothers on infant hepatitis B vaccine (HepB) response

| Variables | Adjusted $\beta$-value | SE | t | P |
|---|---|---|---|---|
| Folic acid supplementation during the first trimester | 194·12 | 64·57 | 3·01 | 0·003 |
| HBV DNA | 16·26 | 75·98 | 0·21 | 0·830 |
| HBeAg | 62·01 | 75·51 | 0·82 | 0·413 |
| Nutrient supplementation during 1 year prior to conception* | −4·74 | 83·70 | −0·06 | 0·955 |
| Other nutrient supplementation during the first trimester* | −5·65 | 93·68 | −0·06 | 0·952 |
| Nutrient supplementation during the second trimester* | −7·48 | 64·54 | −0·12 | 0·908 |
| Nutrient supplementation during the third trimester* | 86·02 | 74·88 | 1·15 | 0·252 |
| Prematurity | 146·73 | 204·05 | 0·72 | 0·473 |
| Birth weight | 155·19 | 111·07 | 1·40 | 0·164 |
| Neonatal anti-HB | 19·95 | 16·54 | 1·21 | 0·229 |
| Infant feeding patterns | −7·71 | 50·06 | −0·15 | 0·878 |

HBV, hepatitis B virus; HBsAg,

* 'Nutrient supplementation (yes) = 1' was that the pregnant woman had taken any of the nutrient supplements, and 'Nutrient supplementation (no) = 0' was that the woman had not taken any of the nutrient supplements. 'Other nutrient supplementation (yes) = 1' was that the pregnant woman had taken any nutrient supplements other than folic acid, and 'Other nutrient supplementation (no) = 0' was that the woman had not taken any nutrient supplements other than folic acid.
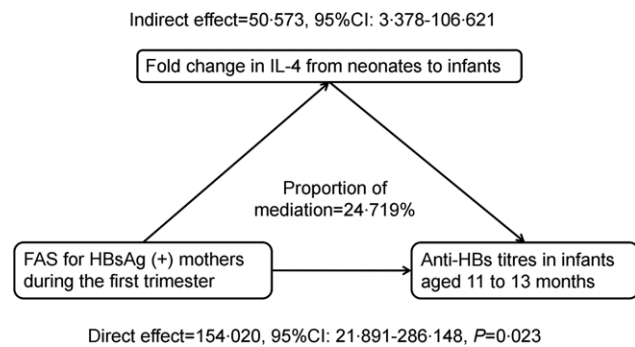


**Fig. 2.** The fold change in IL-4 from neonates to infants as a mediator for association of maternal folic acid supplementation in the first trimester and hepatitis B surface antibody (anti-HB) titres among infants aged from 11 to 13 months. FAS, folic acid supplementation.

*The mediation of the influence of maternal hepatitis B surface antibody during the first trimester (X) on infant hepatitis B surface antibody (Y) by IL-4 alteration from neonates to infants (M)*

We performed the mediation effect analyses presented in Fig. 2 to further assess the role of IL-4 in maternal FAS resulting in higher anti-HBs in infants. We adjusted for the same factors as in the above linear regression model.

The mediation model confirmed the significant association between maternal FAS during the first trimester and fold change of IL-4 from neonates to infants (coefficient = 7·711, 95 % CI: 4·677 to 10·756, $P < 0.001$). Likewise, a significant association between the fold change in IL-4 from neonates to infants and infant anti-HBs was observed in the mediation model

(coefficient = 6·558, 95 % CI: 0·376–12·740, $P = 0.038$). The mediation model yielded a significant positive indirect (mediated) effect of maternal FAS during the first trimester on infant anti-HBs through a fold change in IL-4 (total effect = 204·593, 95 % CI, 66·321, 321·951, $P = 0.003$; direct effect = 154·020, 95 % CI: 21·891, 286·148, $P = 0.023$; indirect effect = 50·573, 95 % CI: 3·378, 106·621). A total of 24·719 % of the total effect was mediated by the fold change in IL-4 from neonates to infants.

## Discussion

Existing information has shown that FAS upregulates infant anti-HB in healthy pregnant women[10,11]. Experiments on animals have indicated that exposure to FA before birth was positively associated with IL-4 levels after birth[17,18]. IL-4 levels in adults are positively associated with anti-HBs[25]. However, we have not known whether and how maternal FAS, infant IL-4 and infant anti-HBs are related, especially in these infants at high risk of hepatitis B infection. As a poor response to HepB in infants born to HBsAg-positive mothers hinders the eradication of the HBV, additional interventions are urgently needed. Our work uniquely discovered that infant anti-HBs could be increased by maternal FAS during the first trimester and that the fold change in IL-4 in infants could mediate this favourable influence.

In the present study, the percentage of FAS during the first trimester was 63·59 % (124/195) in pregnant women with seropositive HBsAg, which is similar to that of healthy pregnant women (47·9 % to 75·6 %) reported in different cities in China[30,31]. The two guidelines differed in timing of FAS. Guidelines from WHO (2012) recommend that pregnant adolescents and adult women take 0·4 mg of FA supplements daily throughout the whole pregnancy[32]. Chinese guidelines (2011) recommend that women supplement 0·4 mg of FA daily from 3 months before conception and throughout pregnancy[33]. The following three new guidelines vary in the timing and dose of FAS. The latest NTD prevention guidelines in China (2017) recommend that women take 0·4 mg or 0·8 mg of FA supplements daily from possible pregnancy to the end of the first trimester[7]. The latest dietary guidelines for Chinese residents (2016) and for pregnant women (2016) recommend that women take 0·4 mg of FA supplements daily throughout the pregnancy[8,9]. None of the guidelines highlight the importance of FAS to pregnant women with HBsAg. Pregnant women with HBsAg pay more attention to the status of hepatitis B disease, but neglect the FAS. Our data revealed that none of the pregnant women strictly followed dietary guidelines for Chinese residents (2011) on the starting time and end time of FAS. We provided the first data on the status of FAS in positive HBsAg-pregnant women. Public health education on FAS needs to be further strengthened in the future. We provide evidence for clinicians to guide FAS in pregnant women with hepatitis B during the first trimester.

The infant immune response to routine childhood vaccinations may be affected by maternal nutritional status during pregnancy[13]. We revealed the benefit of FAS during the first trimester to HBsAg-positive pregnant women on infant anti-HBs, which has not yet been reported. The 95 % CI of infant anti-HB between the FAS

group and no FAS group overlapped, and the $P$ value was still less than 0·05. This phenomenon has been proven to be reasonable by statistical deduction and demonstration of examples[34]. Fortunately, only one research team revealed that FAS before pregnancy and in the first trimester in HBsAg-negative pregnant women was associated with anti-HBs titres after HepB vaccination[10]. We speculate that immune development is the underlying mechanism. FA is involved in the synthesis of purine and pyrimidine and is crucial for fetal development[35]. The immune system begins to develop during the fetal period[36]. This critical period of development has been proven to be very sensitive and fragile to nutritional deficiencies[37]. It is well known that the thymus begins to develop in the first trimester. In early childhood, the thymus is the main site of lymphocyte cloning, so it is the key organ that coordinates adaptive immune development[38]. Another vulnerable period of immune development begins at 8 weeks of gestation, which is the formation of lymphoid cells, precursors of immune cells[39]. Well-developed immune organs and immune cells are believed to aid infants in producing anti-HBs.

We estimated that the fold change of IL-4 from neonates to infants explained 24·719 % of the impact of FAS during the first trimester on infant anti-HBs, elucidating that the impact of FA exposure to fetus upon IL-4 after birth contributed to responsiveness of HepB from a statistical perspective. The available data on animals and adults can explain this novel finding from a biological function perspective. McStay Catrina L *et al*.[40] inferred that there might be an the impact of maternal use of FA supplements on fetal development of the immune system, affecting immune function after birth. Immune cells and cytokines are mobilised after the infant is given HepB, a foreign antigen. The effects of FA on the immune system, especially IL-4, were found in animal experiments, but not in human studies. One study reported that the chromatin tightness of the IL-4 promoter regions was improved, histone methylation in IL-4 promoters was altered and the expression levels of IL-4 in broilers were upregulated after injection of FA in hatching eggs[18], but not mention vaccination. Similarly, when pregnant rats were given a high-FA diet, the levels of IL-4 produced by immune cells were upregulated in the offspring after birth[17]. IL-4 is a Th2-type cytokine that is mainly produced by T cells in humans. The main function of IL-4 is to assist in the maturation and proliferation of B cells[41] and enhance the antigen presentation of B cells. It promotes the transformation of B cells into plasma cells that synthesise antibodies[27]. In addition, it can promote the proliferation and activation of T cells[42], which participate in the T-cell-dependent vaccine response. Although there is no evidence of a relationship between IL-4 and HepB response among infants, a randomised controlled trial disclosed a significant positive correlation between IL-4 and anti-HBs titres among adults aged 18 to 45[24]. A meta-analysis suggested that polymorphisms in the IL-4 gene may play a significant role in determining the response to HepB, especially among Asian populations[25]. Whether maternal FAS influences other vaccine responses in infants by regulating IL-4 remains to be investigated.

Several limitations of our study should be mentioned. Some variables were not evaluated including the status of folate biomarkers in pregnant women and neonates. The bioavailability of natural FA is low, only approximately 60 % of that of synthetic FA[7]. Thus, FA intake from supplements predominates over FA intake from diet alone. Emerging data have shown that FAS in adults increases their own concentration of serum folate[16] and a positive correlation has been found between serum folate in pregnant women and folate in cord blood[43]. Hence, we believe that women who took FA supplements would have high levels of folate biomarkers, and women who did not take FA supplements would have low levels of folate biomarkers. We will collect maternal blood samples in the first trimester to detect folate biomarkers in our new cohort study in the future.

Nonetheless, our work has several strengths. A prospective cohort study is preferable to other observational studies as it provides strong evidence for the temporal order for a cause–effect association. Double entry of the questionnaire ensured the accuracy of the data, minimising the information bias. Moreover, we developed a theory-based concept linking maternal FAS and fold change of IL-4 in infants with favourable outcomes in infant HepB response and used a mediating effect model to test it for the first time. The advantage of the mediation effect model is to understand and explain why and how causality occurs and to reveal the intermediate process between cause and effect.

In summary, FAS during the first trimester by HBsAg-positive mothers ameliorates infant anti-HBs, partly owing to the indirect effects of IL-4 in the offspring. We therefore recommend that HBsAg-positive women continue to take FA supplements from conception until 13 weeks of gestation as it may also help to boost their infant anti-HBs. Our work opens up a new perspective for preventive intervention in infants at high risk of low anti-HBs. This will be expected to improve the herd immunity against HBV in an even better fashion, reducing the continuous accumulation of hepatitis B patients due to the transmission of HBV from generation to generation.

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## Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S000711452200229X

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