

# Breast-feeding, dietary intakes and their associations with subclinical vitamin A deficiency in children in Anhui Province, China

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## Abstract

**Objective:** This study aimed to explore the associations between breast-feeding, dietary intakes and other related factors and subclinical vitamin A deficiency (SVAD) in children aged 0–5 years in an area in China where mild vitamin A deficiency (VAD) is found.

**Methods:** Data were from a population-based cross-sectional study with 1052 children aged 0–5 years. SVAD cases were identified by the indicator of serum retinol  $\leq 20 \mu\text{g dl}^{-1}$ . Breast-feeding status, dietary intakes and other factors were collected through a pre-designed questionnaire.

**Results:** The prevalence of SVAD in children aged 0–5 years was 6.9%. Logistic regression analysis showed that the odds ratio of SVAD for breastfeeding was 3.56 (95% confidence limits (95% CL) 2.17–5.82). After adjustment for sex, age in categories, residence, mother's education, mother's occupation, vitamin A preparation supplements, rank in siblings and diarrhoea, the odds ratio for breastfeeding fell to 2.38 (95% CL 1.13–4.95). The odds ratios for breastfeeding within children aged 1 year were 5.46 (95% CL 2.07–15.03) and 4.6 (95% CL 1.72–12.82) before and after adjustment of other confounders, respectively. The odds ratios for breastfeeding did not show statistical significance within children aged 0 or 2 years. The odds ratios decreased, but remained statically significant after further adjustments for individual dietary factor or all dietary factors.

**Conclusion:** Breast-feeding was a risk factor of SVAD for children, especially for those aged 1 year. The differences in dietary intakes and other established risk factors could not fully explain the increased risk. This finding implies that prolonged breast-feeding alone may not ensure protection of children from VAD in an area with mild SVAD.

## Keywords

Subclinical vitamin A deficiency  
Breast-feeding  
Dietary intakes  
Serum retinol

Vitamin A deficiency (VAD), even subclinical vitamin A deficiency (SVAD), could increase the risks of morbidity and mortality in children<sup>1,2</sup>. At least 100 million of the world's under-fives are vitamin A deficient<sup>1</sup>. Breast milk, vitamin A-rich foods and foods fortified with vitamin A are recommended and used for prevention of VAD<sup>3,4</sup>. Promoting breast-feeding is regarded as the best way to protect babies from VAD in public health campaigns<sup>3,5</sup>. However, several studies reported a high prevalence of VAD in breast-fed children<sup>6,7</sup>. In some developing countries, VAD has been found to be even more common among women than in children<sup>8,9</sup>, and a deficient mother might not give adequate vitamin A to her child because of low concentrations of vitamin A in her breast milk<sup>6,8,10</sup>. Furthermore, a study reported that

lactating mothers, especially those using prolonged breast-feeding, would be less likely to supply complementary foods adequately or in a timely manner for their children<sup>11</sup>, and breast milk alone may not meet the entire needs for a child aged  $>6$  months. Most of the studies that have reported increased risks of SVAD in breast-fed children were from countries or regions where there was severe VAD. Associations of VAD of children with breast-feeding and dietary intakes have not been well characterised in countries where there is mild VAD, for example in China. The purpose of this study was to report the prevalence of SVAD and explore the associations between SVAD and breast-feeding, complementary food intakes and other risk factors in children aged 0–5 years in Anhui Province, China.

## Methods

The cross-sectional study was conducted in December 2002 in Anhui Province, one of the agricultural provinces in the middle east of China, with a population of >60 million. A total of 1097 children aged between 0 and 5 years were recruited for the study using the proportionate-to-population size cluster sampling method from four urban districts in one city and 24 rural villages in four counties. If more than one eligible child lived in a household, investigators randomly selected one for the survey.

Mothers or caregivers were asked to bring the children selected for the survey to the local township hospitals for evaluation. After verbal consent was obtained from children's parents or caregivers, mother or caregivers were interviewed about the child's demographics, breast-feeding and other food consumption in the past week, any vitamin A supplement in the past 6 months and the presence of diseases during the previous 2 weeks. Information on the mother's their demographics, reproductive history and family's socio-economic status was also collected.

Questions about breast-feeding practices were asked: 'Has your child been breastfed in the past week? If "Yes" what type of breast-feeding? Exclusive or non-exclusive breast-feeding?' Children who were breast-fed, and with no supplementation of any type (no water, no juice, no non-human milk and no foods) except for vitamins, minerals and medications, were categorised into the exclusive breast-feeding group<sup>12</sup>; the remainder were classified into the non-exclusive breast-feeding group.

Food consumption in the past week was also assessed using a semi-quantitative food-frequency questionnaire, which was designed to include all important food sources of vitamin A and carotenoids in the typical local diet and used in the national survey for VAD in China in 2001<sup>13</sup>. The questions were 'In the past week, how often did your children eat (or drink) any of following foods: cow's milk or its products, cereals, eggs, pulses or soy foods, red and yellow fruits, dark green leafy vegetables, fish and shrimps, meat and animal liver?' Response categories for every type of foods were none, once or more a week and at least once a day.

Vitamin A supplement intake was determined using the question: 'Has your child had any supplementation of fish-liver oil or other vitamin A preparations in the past 6 months?'

About 0.2 ml of capillary blood was drawn by finger stick for serum retinol determination from all participants in a dark room and placed in a plastic EDTA tube without anticoagulant. Serum was obtained by centrifugation within 30 min. The serum tube was protected from light by wrapping it in aluminium foil, placed in a cold chain box containing packets of dry ice and then frozen at  $-20^{\circ}\text{C}$  within 4 h. All specimens were finally transported on dry

ice to the laboratory of the Capital Institute of Pediatrics and stored at  $-70^{\circ}\text{C}$  until assay. Serum retinol was detected at an emission wavelength of 480 nm and an excitation wavelength of 330 nm with a Shimadzu RF-540 fluorescence detector. The analytical protocols and laboratory quality assurance procedures of the US National Health and Nutrition Examination Survey (NHANES)<sup>15</sup> were followed by this laboratory.

The cut-off value for SVAD was defined as serum retinol  $\leq 20 \mu\text{g dl}^{-1}$  according to criteria recommended by the World Health Organization. This research protocol was reviewed and approved by the Ethical Committee in Anhui Medical University.

We used the  $\chi^2$  test for categorical variable analysis and the Cochran–Armitage method for  $\chi^2$  trend analysis. To control confounding as well as to test the possible interactions between breast-feeding and other factors, we estimated and tested the odds ratios in the logistic model with interaction using dummy coding<sup>14</sup>. We conducted all the data analysis using SAS software version 8.2 (SAS Institute Inc.). All tests were two-sided and were considered significant at 0.05.

## Results

### *Characteristics of the study population*

A total of 1097 children aged 0–71 months were recruited for this study. Forty-five children were excluded due to lack of vitamin A data. Of 1052 children, 72 SVAD cases were found. The prevalence rate of SVAD was 6.9% in all children, and 13.0, 9.1, 5.3, 2.9, 4.3 and 4.7% in children aged 0, 1, 2, 3, 4 and 5 years, respectively.

The breast-feeding rates were 78.33% (159/263), 23.74% (52/219) and 5.12% (11/215) for children aged 0, 1 and 2 years, respectively. The breast-feeding rates declined with age (Cochran–Armitage trend test,  $P < 0.01$ ).

Table 1 shows the characteristics of children with SVAD and non-SVAD. Gender, fever in the previous 2 weeks, and cold, cough or other infection of the respiratory tract in the previous 2 weeks were similar between the two groups.

Significant differences between SVAD and non-SVAD groups were found in age in categories, place of residence, rank among siblings, mother's education, mother's occupation, vitamin A preparation supplementations, and diarrhoea in the previous 2 weeks.

### *The odds ratios of SVAD for breast-feeding in all children and within age categories*

The odds ratio of SVAD for breast-feeding was 3.56 (95% confidence limits (95% CL) 2.17–5.82). After adjustment for sex, age in categories, residence, mother's education, mother's occupation, vitamin A preparation supplements, rank in siblings and diarrhoea, the odds ratio for breastfeeding fell to 2.38 (95% CL 1.13–4.95), but remained statically significant (Table 2).

**Table 1** Characteristics of children with subclinical vitamin A deficiency (SVAD) and non-SVAD in Anhui Province, China, 2002

Characteristics of the children	Non-SVAD n (%)	SVAD† n (%)
Age (years)**		
0	168 (17.1)	25 (34.7)
1	189 (19.3)	19 (26.4)
2	195 (19.9)	11 (15.3)
3	169 (17.2)	5 (6.9)
4	157 (16.0)	7 (9.7)
5	102 (10.4)	5 (6.9)
Sex		
Male	566 (57.8)	43 (59.7)
Female	414 (42.2)	29 (40.3)
Residence**		
Rural	784 (80.0)	68 (94.4)
Urban	196 (20.0)	4 (5.6)
Ranking among siblings**		
First	790 (80.6)	45 (62.5)
Second or more	190 (19.4)	27 (37.5)
Mother's education*		
Junior high school or less	809 (82.6)	67 (93.1)
Senior high school or above	171 (17.4)	5 (6.9)
Mother's occupation**		
Non-farmer	690 (70.4)	63 (87.5)
Farmer	290 (29.6)	9 (12.5)
Vitamin A preparation supplementation**		
Yes	240 (24.5)	6 (8.3)
No	740 (75.5)	66 (91.7)
Fever in the previous 2 weeks		
No	852 (86.9)	57 (79.2)
Yes	128 (13.1)	15 (20.8)
Diarrhoea in the previous 2 weeks**		
No	877 (89.5)	57 (79.2)
Yes	103 (10.5)	15 (20.8)
Cold, cough or other infection of the respiratory tract in the previous 2 weeks		
No	579 (59.1)	43 (59.7)
Yes	401 (40.9)	29 (40.3)

\*  $\chi^2$  test,  $P < 0.05$ .\*\*  $\chi^2$  test,  $P < 0.001$ .† The cut-off value for SVAD was defined as serum retinol  $\leq 20 \mu\text{g dl}^{-1}$ .

We estimated the odds ratios for breast-feeding within age categories using logistic regression with interaction by dummy coding. The odds ratios for breast-feeding did not show statistical significance within age 0 and 2 categories. The odds ratios for breast-feeding within age 1 category were 5.46 (95% CL 2.07–15.03) and 4.6 (95% CL 1.72–12.82) before and after adjustment for other confounders, respectively, and both were statistically significant (Table 2).

The odds ratios for exclusive breast-feeding did not show statistical significance within age 0, 1 and 2 categories, although the odds ratios for exclusive breast-feeding reached 6.29 and 8.93 before and after adjustment for other confounders, respectively, within age 1 category. The odds ratios for non-exclusive breast-feeding within age 1 category were 5.39 (95% CL 1.99–15.1) and 4.36 (95% CL 1.58–12.01), and showed statistical significance before and after adjustment of other confounders, but no statistical significance was found for that within the age 0 and 2 categories (Table 2).

The odds ratios for breast-feeding decreased, but remained statically significant, after further adjustments for individual dietary factors or all dietary factors (data not shown).

### *The effects of dietary intakes on SVAD*

Cochrane–Armitage  $\chi^2$  trend tests showed negative or decreasing trends between the SVAD and the frequencies of food intakes, except for meat and liver. Univariate logistic regression analysis showed that the intakes of milk or its products, eggs, pulses or soy foods, red and yellow fruits, dark green vegetables, fish and shrimps, etc. had protective effects on SVAD (Table 3). After adjustment for breast-feeding, sex, age in categories, rank in siblings, mother's education, mother's occupation, vitamin A preparation supplements and diarrhoea, no food intake was found to have a significant effect on SVAD (Table 3).

### *The effects of other factors on SVAD*

The crude odds ratios of SVAD for residence (4.25, 95% CL 1.53–11.79), mother's education (2.83, 95% CL 1.12–7.13), mother's occupation (2.94, 95% CL 1.44–5.99), breast-feeding, vitamin A preparation supplements (0.28, 95% CL 0.12–0.65), rank in siblings (2.49, 95% CL 1.51–4.12) and diarrhoea (2.24, 95% CL 1.22–4.1) were statistically significant. For those factors, only rank in siblings (odds ratio 2.08, 95% CL 1.79–3.62) remained statistically significant in the full adjusted model including sex, age in categories, residence, mother's education, mother's occupation, mother's occupation, vitamin A preparation supplements and diarrhoea.

## **Discussion**

The prevalence of SVAD in our study population was 6.9%, which was lower than the national average of 11.7%, but comparable with the 5.8% in the coastal areas in China in 2001<sup>13</sup>. Two other studies from Zhejiang and Fujian provinces which are adjacent to Anhui reported that the prevalence of SVAD was 7.8% in children aged 0–5 years and 6.3% in children aged 0–6 years, respectively<sup>15,16</sup>.

We found that breast-feeding in children, especially for those aged 1 year, was associated with an increased risk of SVAD. The effects remained statistically significant after adjustment for sex, age, the order in siblings, mother's education, mother's occupation, vitamin A supplementation and diarrhoea, as well as dietary factors. Univariate logistic regression analysis showed that those who received high, frequent intakes of complementary foods tended to be less likely to have SVAD. However, no food showed a statistically significant effect after adjustment for breast-feeding and other confounders. Residing in rural areas, ranking second or more in the order of siblings, mother's education less than junior high school, mother's occupation of farmer, no vitamin A preparation supplementation in the previous 6 months, and diarrhoea in

**Table 2** Odds ratios of subclinical vitamin A deficiency (SVAD) for breast-feeding in children aged 0–71 months, Anhui Province, China, 2002

	Non-SVAD	SVAD†	Crude OR (95% CL)	Adjusted OR‡ (95% CL)
Breast-feeding§ in two categories				
Non-breast-feeding	800	40	Reference	Reference
Breast-feeding	180	32	3.56 (2.17–5.82)**	2.36 (1.13–4.95)**
Effects within age categories§				
Age 0				
Non-breast-feeding	36	5	Reference	Reference
Breast-feeding	132	20	1.09 (0.41–3.46)	1.0 (0.37–3.24)
Age 1				
Non-breast-feeding	151	8	Reference	Reference
Breast-feeding	38	11	5.46 (2.07–15.03)**	4.6 (1.72–12.82)**
Age 2				
Non-breast-feeding	185	10	Reference	Reference
Breast-feeding	10	1	1.85 (0.10–11.22)	1.69 (0.09–10.48)
Breast-feeding in three categories				
Non-breast-feeding	800	40	Reference	Reference
Exclusive	58	8	2.76 (1.23–6.17)**	1.78 (0.59–5.13)**
Non-exclusive	122	24	3.93 (2.29–6.76)**	2.47 (1.18–5.2)**
Effects within age groups¶				
Age 0				
Non-breast-feeding	36	5	Reference	Reference
Exclusive	55	7	0.92 (0.27–3.30)	0.83 (0.24–2.90)
Non-exclusive	77	13	1.22 (0.42–4.02)	1.12 (0.36–3.47)
Age 1				
Non-breast-feeding	151	8	Reference	Reference
Exclusive	3	1	6.29 (0.29–55.97)	8.93 (0.79–101.37)
Non-exclusive	35	10	5.39 (1.99–15.10)**	4.36 (1.58–12.01)**
Age 2				
Non-breast-feeding	185	10	Reference	Reference
Exclusive	0	0	–	–
Non-exclusive	10	1	1.85 (0.10–11.22)	1.69 (0.19–14.77)

OR – odds ratio; 95% CL – 95% confidence limits.

\*\*  $P < 0.01$ .

† The cut-off value for SVAD was defined as serum retinol  $\leq 20 \mu\text{g dl}^{-1}$ .

‡ Adjusted for breast-feeding, sex, age in categories, rank in siblings, mother's education, mother's occupation, vitamin A preparation supplement, and diarrhoea in the previous 2 weeks.

§ Breast-feeding: children breast-fed in the past week were categorised as breast-feeding, otherwise as non-breast-feeding. For children in the breast-feeding category, those with no supplementation of any type (no water, no juice, no non-human milk and no foods) except for vitamins, minerals and medications were categorised in the exclusive breast-feeding group, otherwise in the non-exclusive breast-feeding group.

¶ Odds ratios were estimated and tested in the logistic model with interaction using dummy coding.

the past 2 weeks were risk factors for SVAD in the univariate analysis; only children ranked second or more in the order of siblings were found to be at risk of SVAD in the fully adjusted logistic regression model.

Our finding regarding the association between breast-feeding and SVAD among children aged 0–5 years was comparable with a recent study of pre-school children in Nigeria, which shows that serum retinol deficiency was highest in those who were breast-feeding<sup>7</sup>. Another recent study in Kenya indicated that the risk of VAD in breast-fed infants older than 6 months was high<sup>6</sup>. Generally, for a healthy and well-nourished mother, even exclusive breast-feeding for her baby during the first 6 months of life would be enough to provide all the nutrients including vitamin A<sup>17</sup>. Breast milk also protects babies from diarrhoea and acute respiratory infections that are considered to be the risk factors of VAD<sup>3,18,19</sup>. However, for malnourished mothers, breast-feeding alone may not meet the child's needs for vitamin A. Data reported over the past decade indicate that in developing countries, VAD may be even more common among women than in children, and deficient mothers may not provide sufficient vitamin A in their breast milk<sup>8,9</sup>. Low breast milk concentrations of vitamin A were reported in lactating mothers in several studies<sup>8–10</sup>. Another consideration is that if mothers failed to supply an adequate supplementary diet, the risk of SVAD

for the breast-fed child would increase. Fawzi *et al.* reported that breast-fed children in the first 2 years of life were more likely to have a low dietary vitamin A intake compared with non-breast-fed children in the Sudan<sup>11</sup>. High fertility with prolonged breast-feeding would further heighten the risk of VAD for both mothers and their children. Concentrations of vitamin A may not increase in accordance with the growth of children or with increased duration of lactation<sup>10</sup>. Our study also found that children ranked second or above among siblings were more likely to have SVAD. Therefore, latent factors underlying breast-feeding practice or breast milk should be considered as an explanation of the association between breast-feeding and SVAD in this study.

Because of increasing demands for vitamin A for rapid growth, breast milk alone may be not able to meet the entire needs of children aged 6 months or more. Other dietary sources of vitamin A should be added in their diets<sup>6</sup>. Vitamin A-rich foods from both animal and plant sources are recommended to be included in the diet for prevention of VAD<sup>3</sup>. Our study showed that higher frequency intakes of milk, eggs, red and orange fruits, pulses and related products, dark green leafy vegetables, fish and shrimps were associated with reduced risks of SVAD, but not of meat and liver before adjustment for confounders. After adjustment for breast-feeding and

**Table 3** Odds ratios of subclinical vitamin A deficiency (SVAD) in association with dietary intakes among children aged 0–5 years in Anhui Province, China, 2002

Frequencies of intake of foods†	Non SVAD <i>n</i> (%)	SVAD† <i>n</i> (%)	Crude OR (95% CL)	Adjusted OR (95% CL)§
<b>Milk or related products**</b>				
≥ Once a day	350 (35.7)	16 (22.2)	0.47 (0.26–0.83)	0.67 (0.35–1.26)
≥ Once a week	131 (13.4)	7 (9.7)	0.54 (0.24–1.23)	0.72 (0.31–1.65)
None	499 (50.9)	49 (68.1)	Reference	Reference
<b>Cereals**</b>				
≥ Once a day	860 (87.8)	55 (76.4)	0.40 (0.21–0.79)	0.7 (0.31–1.57)
≥ Once a week	44 (4.5)	5 (6.9)	0.72 (0.24–2.18)	0.94 (0.29–3.06)
None	76 (7.8)	12 (16.7)	Reference	Reference
<b>Pulses and soy foods**</b>				
≥ Once a day	234 (23.9)	9 (12.5)	0.28 (0.13–0.60)	0.45 (0.2–1.01)
≥ Once a week	522 (53.3)	32 (44.4)	0.44 (0.26–0.74)	0.65 (0.37–1.16)
None	224 (22.9)	31 (43.1)	Reference	Reference
<b>Eggs**</b>				
≥ Once a day	445 (45.4)	26 (36.1)	0.44 (0.25–0.78)	0.69 (0.37–1.30)
≥ Once a week	347 (35.4)	21 (29.2)	0.46 (0.25–0.83)	0.61 (0.32–1.17)
None	188 (19.2)	25 (34.7)	Reference	Reference
<b>Red and yellow fruits**</b>				
≥ Once a day	333 (34.0)	18 (25.0)	0.42 (0.22–0.80)	1.01 (0.48–2.14)
≥ Once a week	459 (46.8)	30 (41.7)	0.51 (0.29–0.90)	0.78 (0.41–1.47)
None	188 (19.2)	24 (33.3)	Reference	Reference
<b>Green vegetables**</b>				
≥ Once a day	634 (64.7)	39 (54.2)	0.44 (0.25–0.77)	0.89 (0.44–1.80)
≥ Once a week	195 (19.9)	12 (16.7)	0.44 (0.21–0.93)	0.88 (0.39–2.02)
None	151 (15.4)	21 (29.2)	Reference	Reference
<b>Fish**</b>				
≥ Once a day	0	0	–	–
≥ Once a week	646 (65.9)	34 (47.2)	0.46 (0.29–0.75)	0.78 (0.46–1.34)
None	334 (34.1)	38 (52.8)	Reference	Reference
<b>Meat</b>				
≥ Once a day	190 (19.4)	9 (12.5)	0.54 (0.24–1.22)	1.34 (0.54–3.33)
≥ Once a week	560 (57.1)	43 (59.7)	0.88 (0.51–1.53)	1.34 (0.54–3.33)
None	230 (23.5)	20 (27.8)	Reference	Reference
<b>Liver</b>				
≥ Once a day	0	0	–	–
≥ Once a week	313 (31.9)	17 (23.6)	0.66 (0.38–1.15)	0.6 (0.14–2.63)
None	667 (68.1)	55 (76.4)	Reference	Reference

OR – odds ratio; 95% CL – 95% confidence limits.

\*\* Cochran–Armitage  $\chi^2$  trend tests,  $P < 0.001$ .† The cut-off value for SVAD was defined as serum retinol  $\leq 20 \mu\text{g dl}^{-1}$ .

‡ Frequencies of intake of foods refers to the frequencies of food intakes in the past week, and response categories for every type of foods were none, once or more a week and at least once a day.

§ Adjusted for breast-feeding, sex, age in categories, rank in siblings, mother's education, mother's occupation, vitamin A preparation supplement, and diarrhoea in the previous 2 weeks.

other factors, no significant effect on SVAD was shown for those foods. In addition, the difference in risk of SVAD between breast-fed children and non-breast-fed children could not be fully explained by other dietary intakes. This may be partially due to the identical low consumption of expensive animal foods such as meat, liver and fish in the study communities. For plant sources of foods, such as fruit and vegetables, the bioavailability of pro-vitamin A carotenoids was poor (the bioavailability of  $\beta$ -carotene from vegetables and carrots was, on average, only a third of that of  $\beta$ -carotene in oil)<sup>20</sup>; therefore, the increased amount or frequencies of those foods may not ensure an obvious improvement in vitamin A status. Finally, the small numbers of SVAD cases in the study offered limited power for the test, while controlling confounders and covariates and the semi-quantitative food-frequency questionnaire used for this study only provided

information about serving times of food categories but not exact quantities and specific names of foods.

The limitation of this study was that information on the intensity of breast-feeding which would provide further stronger evidence for the casual effects for breast-feeding was not collected. Nevertheless, this study provided results showing that breast-feeding, independently of other perceived factors, was associated with increased risk of SVAD in the children, especially in those with prolonged breast-feeding.

In conclusion, our study found breast-feeding in children, especially for those aged 1 year, could not ensure their protection from SVAD. This finding will help us to pay more attention to children with prolonged breast-feeding in the public health campaigns against VAD even in the areas where there is mild VAD, and consider more comprehensive measures to improve the vitamin A

status in children besides breast-feeding. Furthermore, in order to understand the underlying reasons for the negative association between breast-feeding and SVAD, further research is needed to determine vitamin A status in lactating mothers, the retinol levels of their breast milk and their relationships to SVAD in this province.

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