Daily Oral Low-Dose Vitamin A Supplementation Increases the Retinol Levels in Serum and Breast Milk of Lactating Mothers and Has No Effect on Infant Health Status: A Randomized Placebo-Controlled Trial in China

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Research

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Abstract

Background: Vitamin A supplementation has been advocated as a potential strategy to improve the vitamin A status of lactating mothers and infants. In China, vitamin A supplements are readily available in the form of daily oral low doses. However, the existing clinical trials are limited to single or two high-dose maternal administration.

Objective: We aimed to evaluate the effects of the daily oral low-dose vitamin A supplementation on the retinol levels in serum and breast milk of lactating mothers and the health status of infants in China.

Methods: Lactating mothers who met the inclusion criteria and planned to continue exclusive breast-feeding were randomly assigned to receive either daily oral vitamin A and D drops (one soft capsule of 1800IU vitamin A and 600IU vitamin D_2), or a matching placebo for 2 months. Before and after the intervention, the dietary intake was investigated by instant photography, and the retinol concentration in maternal serum and breast milk was determined by UPLC. During the trial, the health status of infants was diagnosed by pediatrician or reported by lactating mothers.

Results: 245 participants completed the study with 117 in supplementation group and 128 in control group. After the 2-month intervention, maternal serum retinol concentration increased in supplementation group with no change in control group. Although breast milk retinol concentrations decreased significantly in both groups, the decrease in supplementation group was significantly less than that in the control group. However, maternal vitamin A supplementation was not associated with lower risks of infant febrile illness, respiratory tract infection, diarrhea and eczema respectively.

Conclusion: Daily oral low-dose vitamin A supplementation is helpful to improve the maternal vitamin A status and can also play a positive role in vitamin A status of infants through breast milk.

Highlights

- This study evaluated the effects of the daily oral low-dose vitamin A supplementation on the retinol levels in serum and breast milk of lactating mothers and the health status of infants.
- Our results indicated that oral low-dose vitamin A supplementation is helpful to improve the maternal vitamin A status and can also play a positive role in vitamin A status of infants through breast milk.
- Further testing of micronutrients in breast milk is needed to confirm our hypothesis.

1. Introduction

Vitamin A is an essential nutrient, which is extremely important for maintaining normal vision, gene expression, reproduction, embryonic development, growth and immune function [1]. The human body cannot synthesize this nutrient, so it should be exogenously provided. When dietary intake is low for a long time, there will be vitamin A deficiency (VAD). This deficiency remains a global public health problem. Compared with ordinary adults, lactating mothers and infants are in special physiological

period, so they need more vitamin A and will face greater risk of VAD. In China, the serum retinol concentration of about 2.96 million (17.90%) woman of childbearing age [2] and 9.08 million (59.26%) infants aged 0.5-2 years is lower than 1.05 μmol/l [3]. Although the data of vitamin A of infants within 6 months of age is relatively limited, this is enough to show that the vitamin A nutritional status of lactating mothers and infants in China is not ideal.

Although the Chinese Nutrition Society has established new dietary guidelines in 2016, which emphasizes increasing the intake of vitamin A rich foods to improve the nutritional status of lactating mothers [4], the dietary survey of Chinese lactating mothers completed by our research group in 2018 showed that the dietary vitamin A intake of this population is still lower than the recommended value, especially in rural areas [5]. The dietary source of vitamin A is limited, mainly animal liver and dark-green leafy vegetables and fruits. Therefore, it is very important to encourage lactating mothers to eat animal liver once or twice a week. But if a lactating mother does not eat these foods, her vitamin A status may only be available through other forms of supplementation. In China, it is not popularized to strengthen vitamin A in staple food or condiment, and it is relatively easy to obtain nutrient supplements. Vitamin A supplements were viewed as efficient and convenient way to achieve the greatest reductions in the prevalence and consequences of VAD [6]. However, it was reported that only a small number of lactating mothers used nutrient supplements including low-dose levels of vitamin A (563, 1350, 1500 or 2400 IU) per day compared to the pregnant women [7] [8]. Even in urban areas, the proportion of lactating mothers using multivitamin supplements is only 3.1%, not to mention that in rural areas, the proportion of this population is even less [9]. So far, many studies have evaluated the effect of vitamin A supplementation in lactating mothers, but they all involve single or two high-dose maternal administration (single dose 200000 IU, 300000 IU or 400000 IU) [10] [11]. It has not been reported whether daily low-dose vitamin A supplementation can improve the nutritional status of vitamin A in lactating mothers in China, but this is very important, which is an urgent problem to be solved.

For the general population, serum retinol level is an important indicator of vitamin A nutritional status. However, for mothers in exclusive breastfeeding stage, in addition to serum retinol level, breast milk retinol level can also be used as an indicator of vitamin A status of both lactating mothers and infants. Because even if the mother is well nourished during pregnancy, the infant has very little retinol reserves at birth (about 5 μmol). Breast milk is the only source of vitamin A in exclusively breastfed infants until complementary foods are added in the first six months of life [12]. Therefore, vitamin A in breast milk is essential to meet the baby's needs and liver accumulation before weaning. Previous studies have shown that breastfeeding infants fed by vitamin A deficient lactating mothers are susceptible to the results of vitamin A deficiency, resulting in xerophthalmia, anemia and weaken iron metabolism, growth retardation, increased infectious morbidity, suppressed immune response, increased risk of respiratory tract infections and diarrhea-related mortality [13]. Therefore, it is necessary to study whether the daily low-dose vitamin A supplementation can affect the retinol levels in serum and breast milk of lactating mothers and the health status of infants.

2. Materials And Methods

2.1. Participants

From December 2018 to August 2019, lactating mothers at 30-45 days of postpartum had their first postpartum examination in Maternal and Child Health and Family Planning Service Center of Jiangning district in Nanjing city. Among them, mothers aged 20-40 years were invited for screening if they had delivered full-term (gestational age≥37 weeks) singleton healthy infants, had been continuously successfully lactating, and planned to continue exclusive breast-feeding for at least 2 months. Mothers with metabolic diseases such as hypertension and diabetes (including gestational diabetes), clinically diagnosed nutritional disorders (anemia, osteoporosis, iodine deficiency goiter, et al.), mastitis and other breast diseases, disability, mental disorder, infectious disease, any weakening or debilitating condition, or history of alcohol consumption, drug abuse and smoking were not be recruited. Meanwhile, infants with febrile illness, respiratory tract infection, intestinal infection, milk malabsorption, allergy and other diseases and receiving drug treatment were also not recruited. In addition, we excluded mothers who were involved in other studies on food restriction, nutrition intervention, drug intervention, and those who were currently taking dietary supplementations. All lactating mothers provided written informed consent. The trial was approved by Ethics Committee of Nanjing Medical University and registered at clinicaltrials.gov as ChiCTR 1800020179.

2.2 Study design

This study was a parallel-group, partially double-blind, randomized, placebo-controlled trial. Participants who met the inclusion criteria were randomly assigned to either the supplementation group, in which they received oral daily oral vitamin A and D drops (one soft capsule of 1800IU vitamin A and 600IU vitamin D2, Zhejiang hailisheng Pharmaceutical Co., Ltd, China), or control group, in which they received placebo capsules of similar appearance that were continued daily for a period of 2 months. Participants were blinded to group assignment during trial, while trial personnel were aware of it.

Baseline characteristics of both groups were obtained through questionnaire interview and confirmed by maternal and infant physical examination manual and clinical records, including maternal age, maternal height and weight at enrollment and at the end of trial, maternal education level, gestational age, parity, delivery way, maternal past medical history, maternal and infant clinical medication, and infant month age, body length, weight and head circumference at enrollment and at the end of trial.

All participants were required to provide a three-day dietary record, 2 ml blood samples and 10~15 ml breast milk samples at the time of enrollment and at the end of the trial, respectively. The dietary record of lactating mothers was completed by instant photography, and the retinol concentration in maternal serum and breast milk was determined by ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC). During the trial, the health status of infants was diagnosed by pediatrician and/or reported by lactating mothers, including the incidence of febrile illness, respiratory tract infections, diarrhea and eczema.

2.3. Dietary data collection, calculation and evaluation

All participants were asked to complete two 3-day dietary records by instant photography within one week after enrollment and one week before the end of trial, respectively. As reported before, the instant photography, developed by our team, is more convenient and accurate than the 24h- dietary recalls [14].Specifically, before each meal, the food (rice, vegetables, soup, etc.) of lactating mothers should be put into flat tableware separately to ensure that they are not mixed with the food of other family members. Then, the food is placed in the red area of the two-dimensional background (scale with 1cm × 1cm). A note was suggested to put on the background next to the food, indicating the name of the food and the ingredients of the food mix. After that, the food was photographed from directly above, front 45 degrees and back 45 degrees, respectively, and the whole red area of background should appear in photographs. After the meal, the same method is used to photograph the unfinished food. At last, the food photos of lactating mothers were sent to the assessors. The food photos were compared with the food atlas of instant photography to estimate the intake of each food [15]. Primary data obtained from the food photos were double entered into the EpiData software by two trained assessors to verify the accuracy. The daily food consumption, energy and nutrient intake were calculated according to China Food Composition Tables (6th edition) [16]. Data of energy and nutrient intake were imported to Microsoft Excel for statistical analysis. Based on the 2013 Chinese Dietary Reference Intakes [17] the measurements of dietary vitamin A were based on the recommended nutrient intake (RNI).

2.4. Sample collection and retinol concentration detection

Maternal blood and milk samples were collected at enrollment and the end of trial. For the evaluation of serum retinol, 2 ml venous blood from lactating mothers was collected into vacutainer tubes and carried to the laboratory under protection from light where the serum obtained was separated by centrifugation and frozen at -70 ℃ until the analysis. For the evaluation of breast milk retinol, 10~15 ml breast milk from lactating mothers was collected between 07:30 and 09:00 hours by automatic breast pump, with no preference for one breast, into polypropylene tubes protected from light, carried to the laboratory and frozen at -70 ℃ until the analysis.

Serum samples were treated with ethanol to precipitate proteins, and then extracted twice with hexane. Retinyl acetate (Sigma Chemical Corp., St. Louis, MO) dissolved in ethanol was used as an internal standard to determine extraction efficiencies. The hexane layers were pooled and evaporated to dryness with nitrogen, and then redissolved in 200 μL methanol: methylene dichloride (3:1, by vol). Breast milk samples were saponified with alcohol and potassium hydroxide, washed with 100 g NaCl/L, and extracted in hexane. Retinyl acetate was then added and the extract was washed with water and redissolved in methanol. Retinol concentrations were then determined by UPLC (Agilent 1290) by third party testing agency (Suzhou Harmony Health Medical Diagnostics Co., Ltd, Jiangsu Province, China) as described before [3] [18]. This method has intra-assay coefficients of variation (C.V.) of 5%. Retinol concentrations were expressed in μmol/L. Previous studies have suggested serum values ≤ 1.05 μmol/L indicated marginal vitamin A deficiency, while breast milk values ≤ 1.39 μmol/L indicated low retinol

concentration in breast milk [17]. In this study, this standard was used to judge whether the vitamin A in maternal serum and breast milk at different stages met the needs of lactating mothers and their infants.

2.5. Quality control

The questionnaire used to obtain the baseline characteristics was reviewed and revised by experts to ensure its scientificity. All the researchers were well trained on the technical aspects of the trial before the participants were recruited. Researchers on the spot explained to the participants how to record the diet and provided the participants with the introduction of the instant photography, including description of the method and the video of the specific operation. The responses to baseline characteristics and dietary information were retrieved and reviewed in time. The errors were corrected to ensure the integrity and validity of the results. Blood and breast milk samples were collected in strict accordance with the standard sampling methods and were transported from the hospital to the -70 ℃ refrigerator of the laboratory using a low temperature storage box. The same instrument and the same batch of reagents were used to detect the retinol concentration in serum and breast milk. The primary data were double entered into the Epidata software by two trained researchers to verify the accuracy. In the stage of statistical analysis, the appropriate statistical methods were selected strictly according to the statistical requirements and data types, and the influence of potential confounding factors was controlled by multivariable analysis.

2.6 Sample size and statistical analysis

At present, the literature on the effect of low-dose vitamin A intervention on breast milk composition in China is relatively limited. Therefore, we mainly make sample estimation based on whether the dietary vitamin A intake of Chinese lactating mothers reaches the RNI level of vitamin A. As the intake of dietary vitamin A fluctuates greatly in the population, it is not suitable to calculate the sample size by mean and standard deviation, but by rate. Therefore, the sample size was calculated by the following formula:

According to the survey data of dietary vitamin A intake of lactating mothers 2-4 months after delivery in three cities, which were similar to Nanjing in geographical location and economic level [7], the effective rate in the control group was 0.136. We assumed that after intervention with vitamin A supplements, the effective rate in the supplementation group was 3 times higher than that in the control group, that is 0.408. By substituting $\alpha = 0.05$ and $\beta = 0.1$ into the formula, the minimum sample size per group contained 54 participants. Since lactating mothers need to recover their body and take care of their babies, the rate of loss of follow-up is high. Assuming that 20% of the subjects may lose the follow-up during the follow-up period, the sample size of each group will be adjusted to $n1 = n2 = 65$.

Statistical analysis of all data was performed using Statistical Package for the Social Sciences V.22. There was statistically significant at level of 0.05 ($P<0.05$). Normally distributed continuous variables were expressed as the mean and standard deviation (SD); non-normally distributed continuous variables data were expressed as median and inter-quartile range (IQR). Categorical variables were expressed as the frequency (n) and percentage (%). Independent samples t test or chi square ($\chi 2$) test were used to

compare the normally distributed continuous variables and categorical variables related to baseline characteristics in the two groups respectively. Mann Whitney U test was used to compare the difference of energy and nutrients intake between the two groups. At the time of enrollment and at the end of the trial, independent samples t test was used to analyze the differences of retinol concentrations in maternal serum and breast milk between the two groups, while paired t test was used to analyze the intra group differences before and after the intervention. The associations between vitamin A intervention and retinol concentrations in maternal serum and breast milk were examined by multiple linear regressions, while the associations between vitamin A intervention and health status of infants were examined using logistic regressions. The covariates considered mainly include maternal age, BMI at the end of trial, education, gestational weeks, parity, delivery way, dietary vitamin A intake, diet composition (fat: carbohydrate ratio), dietary energy, protein and fiber intake, infant age, weight, length, and head circumference.

3. Results

3.1 Participants and baseline characteristics

The participant flowchart is presented in Fig. 1. A total of 412 participants were screened for eligibility, and 294 participants met the inclusion criteria and were randomly assigned to supplementation (150 participants) and control (153 participants) groups. By the end of the trial, 245 participants completed the study (117 in the supplementation group and 128 in the control group). During the two-month intervention period, 15 participants stopped intervention due to family problems and 44 participants were lost to follow-up, of which 18 were due to early weaning and 16 were due to moving away. After statistical analysis, there was no significant difference between the two groups in baseline characteristics of subjects who lost follow-up.

Baseline characteristics of both groups completing the trial was presented in Table 1. The mean maternal age was 29.8 years old, and the mean maternal BMIs at enrollment and the end of the trial were 23.5 and 23.6 kg/m2, respectively. Most of them have good education, with an average of 15.66 years. The mean gestational age of these participants was 39.2 weeks, 65.7% of them were primiparas, and 59.6% were spontaneous labor. The characteristics of the participants were similar in the two groups except for the maternal BMI at the end of trial

Table 1

Comparison of baseline maternal characteristics between supplementation and control groups*

Characteristics	Supplementation (n = 117)	Control (n = 128)	*P*-value
Age, years	30.1 ± 4.1	29.5 ± 3.4	0.16
BMI at enrollment, kg/m^2	23.48 ± 2.90	23.28 ± 2.49	0.34
BMI at the end of trial, kg/m^2	24.03 ± 2.7	23.04 ± 2.4	0.03
Education, years	15.44 ± 2.99	15.22 ± 3.63	0.58
Gestational age, weeks	39.30 ± 1.27	39.49 ± 1.25	0.24
Primiparous, n (%)	73 (62.4)	88 (68.8)	0.30
Spontaneous labor, n (%)	62 (53.0)	84 (65.6)	0.05

Values are Means ± SDs for continuous variables or number and percentage [n (%)] for categorical variables.

P-values were assessed using student's *t* test or χ^2 test.

Supplementation group: in the form of 1800 IU retinol, control group: in the form of placebo.

BMI: body mass index

3.2 Maternal daily dietary energy and nutrient intakes between supplementation and control groups at enrollment and the end of trial

In general, Table 2 shows that no significant variation was observed for dietary energy and nutrient intakes between supplementation and control groups at enrollment and the end of trial. Specifically, at the time of enrollment, the median dietary energy intake of supplementation and control groups were 2189.9 kcal/d and 2210.2 kcal/d, respectively. The dietary intakes of energy producing nutrients (carbohydrate, fat and protein) were also similar between the two groups. After calculation, the fat to carbohydrate ratio was 0.30 in supplementation group and 0.34 in control group, and the difference was not statistically significant. Moreover, in supplementation and control groups, the intake of dietary vitamin A was 711.5 μg RAE/d and 732 μg RAE/d, respectively. Compared with the RNI value of vitamin A, the number of participants whose dietary vitamin A intake was lower than the RNI value was 82.1% and 85.9% respectively, and the difference was not statistically significant. At the end of the trial, the median dietary energy intakes were 2019.5 kcal/d and 2210.2 kcal/d in the supplementation and control groups, respectively. After the two-month period, the dietary intakes of energy producing nutrients (carbohydrate, fat and protein) and the fat to carbohydrate ratio remained similar between the two groups. The median dietary intake of vitamin A was still comparable between the supplementation and control groups (706.6 μg RAE/d vs. 783.9 μg RAE/d), and the dietary vitamin A intake of 82.1% and 85.9% participants was below the RNI value, respectively.

Table 2
Comparison of maternal daily dietary energy and nutrient intakes between supplementation and control groups at enrollment and the end of trial

Energy and nutrients	Supplementation (n = 117)	Control (n = 128)	*P*-value
At enrollment			
Energy (kcal/day)	2189.9 (1878.1, 2620.8)	2210.2 (1943.9, 2586.8)	0.74
Carbohydrate (g/day)	264.8 (224.8, 325.9)	258.8 (224.8, 314.5)	0.94
Fat (g/day)	85.0 (64.5, 97.4)	83.0 (67.2, 101.4)	0.27
Fat to carbohydrate ratio	0.30 (0.24, 0.39)	0.34 (0.23, 0.40)	0.34
Protein (g/day)	102.8 (87.4, 128.9)	102.3 (81.3, 114.6)	0.94
Vitamin A (μg RAE/day)	711.5 (521.6, 1143.3)	732 (502.5, 1078.7)	0.56
At the end of trial			
Energy (kcal/day)	2019.5 (1754.3, 2312.4)	2210.2 (1943.9, 2586.8)	0.50
Carbohydrate (g/day)	245.9 (191.1, 295.9)	264.8 (224.8, 325.9)	0.65
Fat (g/day)	69.8 (53.4, 88.1)	84.9 (64.5, 97.4)	0.27
Fat to carbohydrate ratio	0.29 (0.24, 0.39)	0.28 (0.22, 0.35)	0.95
Protein (g/day)	83.67 (73.96, 108.2)	81.94 (68.1, 101.5)	0.94
Vitamin A (μg RAE/day)	706.6 (567.4, 1097.6)	783.9 (477.6, 1066.9)	0.77
Values are median (P25, P75).			

P-values were assessed using Mann-Whitney U test.

Supplementation group: in the form of 1800 IU vitamin A, control group: in the form of placebo.

RAE: retinol activity equivalent.

3.3 Effect of vitamin A supplementation on maternal serum retinol concentration at the end of trial

As shown in Fig. 2A, at the time of enrollment, the baseline maternal serum retinol concentrations in supplementation and control groups were comparable with 5.3% and 4.7% of the participants having a serum retinol concentration ≤ 1.05 μmol/L (defined as marginal vitamin A deficiency) respectively. After the 2-month intervention, maternal serum retinol concentration increased significantly in supplementation group (from 1.65 ± 0.41 μmol/L to 1.81 ± 0.44 μmol/L; P = 0.00) with no change in control group (from 1.66 ± 0.40 μmol/L to 1.66 ± 0.41 μmol/L; P = 0.98). Meanwhile, marginal vitamin A deficiency rate decreased significantly with vitamin A supplement (from 6.0–0.9%; P = 0.03) with no change with the placebo (from 4.7–8.6%; P = 0.21). The difference of serum retinol concentration before and after the intervention was then calculated, and the results showed that the difference between the two groups was

statistically significant, accompanied by the significant difference of marginal vitamin A deficiency between the two groups.

As shown in Table 3, maternal serum retinol concentration increased significantly in the supplementation group compared to that in the control group (β = 0.170, 95%CI = 0.060–0.274, P = 0.002). In the multivariable analysis that was adjusted for baseline characteristics (maternal age, BMI at the end of trial, education, gestational weeks, parity, and delivery way), and factors that may have affected vitamin A status including dietary vitamin A intake, and diet composition (fat: carbohydrate ratio), differences between the two groups in changes in maternal serum retinol concentration remained significant. In addition, the intake of dietary energy, protein and fiber were also considered on the basis of the previous model, and they did not alter the results (some data not shown)

Table 3
Association between maternal vitamin A supplementation and retinol concentration in maternal serum and breast milk at the end of trial

Dependent variables and models	R^2	β	**β (95% CI)**	***P*-value**
Serum retinol concentration, μmol/L				
Model 1	0.038	0.170	0.060–0.274	0.002
Model 2	0.507	0.168	0.087–0.249	0.000
Model 3	0.508	0.171	0.089–0.252	0.000
Breast milk retinol concentration, μmol/L				
Model 1	0.053	0.146	0.077–0.254	0.001
Model 2	0.319	0.144	0.064–0.223	0.000
Model 3	0.323	0.147	0.068–0.277	0.000
Model 1 was unadjusted.				

Model 2 was adjusted for maternal age, BMI at the end of trial, education, gestational weeks, parity, delivery way, dietary vitamin A intake, and diet composition (fat: carbohydrate ratio).

Model 3 was adjusted for maternal age, BMI at the end of trial, education, gestational weeks, parity, delivery way, dietary vitamin A intake, diet composition (fat: carbohydrate ratio) and dietary energy intake.

P-values were assessed using a multivariable linear regression model for differences between groups with adjustment for covariates.

CI: confidence interval

3.4 Effect of vitamin A supplementation on maternal breast milk retinol concentration at the end of trial

As shown in Fig. 2B, at the time of enrollment, the baseline maternal breast milk retinol concentrations in supplementation and control groups were comparable with 47.9% and 42.2% of participants having a breast milk retinol concentration < 1.39 μmol/L (defined as low retinol concentration in breast milk) respectively. After the 2-month intervention, breast milk retinol concentrations decreased significantly in both supplementation (from 1.56 ± 0.70 μmol/L to 1.02 ± 0.37 μmol/L; P = 0.00) and control (from 1.56 ± 0.56 μmol/L to 0.85 ± 0.34 μmol/L; P = 0.00) groups. Meanwhile, the proportion of mothers with low retinol breast milk also increased significantly in both supplementation (from 47.9–85.5%; P = 0.00) and control (from 42.2–93.8%; P = 0.00) groups. The difference of breast milk retinol concentration before and after the intervention was then calculated, and the results showed that the decrease in supplementation group was significantly less than that in the control group. (-0.53 ± 0.69 μmol/L, vs. -0.71 ± 0.63 μmol/L; P = 0.00) accompanied by the significant difference of low retinol breast milk between the two groups.

As shown in Table 3, maternal breast milk retinol concentration increased significantly in supplementation group compared to that in the control group (β = 0.144, 95%CI = 0.064–0.223, P = 0.000). In the multivariable analysis that was adjusted for baseline characteristics (maternal age, BMI at the end of trial, education, gestational weeks, parity, and delivery way), and factors that may have affected vitamin A status including dietary vitamin A intake, and diet composition (fat: carbohydrate ratio), differences between the two groups in changes in maternal breast milk retinol concentration remained significant. In addition, the intake of dietary energy, protein and fiber were also considered on the basis of the previous model, and they did not alter the results (some data not shown).

3.5 Effect of maternal vitamin A supplementation on the health status of infants during the trial

During the 2-month follow-up period, the incidence of febrile illness, respiratory tract infection, diarrhea and eczema in infant of both two groups were analyzed. The incidence rate of above diseases in the supplementation group was 8.5%, 7.7%, 6.8% and 12% respectively, and that in the control group was 5.5%, 14.1%, 10.9% and 9.3% respectively. Statistical analysis of the data showed that maternal vitamin A supplementation was not associated with lower risks of infant febrile illness (RR = 1.62, 95%CI = 0.59–4.39, P = 0.35), respiratory tract infection (RR = 0.61, 95%CI = 0.31–1.23, P = 0.16), diarrhea (RR = 0.60, 95%CI = 0.24–1.48, P = 0.27) and eczema (RR = 0.90, 95%CI = 0.41–1.99, P = 0.80) respectively. As shown in Table 4, in the multivariable analysis that was adjusted for infant age, weight, length, and head circumference, differences between groups in changes in risks of infant diseases (febrile illness, respiratory tract infection, diarrhea, and eczema) remained nonsignificiant.

Table 4

Association between maternal vitamin A supplementation and the health status of infants during the trial

Disease patterns	Supplementation (n = 117)	Control (n = 128)	RR (95% CI)	*P*-value[1]	Adjusted RR (95%CI)	P-value[2]
Febrile illness	10 (8.5)	7 (5.5)	1.62 (0.59–4.39)	0.35	1.63(0.59–4.52)	0.35
Respiratory tract infection	9 (7.7)	18 (14.1)	0.61 (0.31–1.23)	0.16	0.60(0.29–1.22)	0.16
Diarrhea	8 (6.8)	14 (10.9)	0.60 (0.24–1.48)	0.27	0.59(0.29–1.22)	0.26
Eczema	14 (12.0)	12 (9.3)	0.90 (0.41–1.99)	0.80	0.87(0.39–1.95)	0.73
Values are number and percentage [n (%)] unless otherwise specified.						
P-values were assessed using a multivariable logistic regression model for differences between groups with adjustment for covariates.						
[1] *P*-values were for RR; [2] *P*-values were for adjusted RR.						
Adjusted RR was adjusted for infant age, weight, length, and head circumference.						
RR: relative risk, CI: confidence interval.						

4. Discussion

This randomized placebo-controlled trial examined the effect of daily oral 1800 IU vitamin A supplementation for 2 months on the retinol levels in serum and breast milk of lactating mothers and the health status of infants. The results showed that low-dose vitamin A intervention significantly increased the serum retinol concentration of lactating mothers and alleviated the decrease in the breast milk retinol concentration. However, maternal low-dose vitamin A supplementation was not associated with lower risks of infant diseases (febrile illness, respiratory tract infection, diarrhea, and eczema).

Before evaluating the effectiveness of vitamin A supplementation, it is necessary to compare dietary vitamin A levels. Previous studies mostly used 24 h- dietary recalls to obtain dietary vitamin A intake information [19] [20], while our study used instant photography to record the 3-day dietary intake of lactating mothers. Our previous study found that, compared with the conventional 24 h-dietary recalls, the instant photography can obtain the food consumption and nutrient intake data closer to the data obtained by weighing method [14]. Therefore, the results of dietary vitamin A we get are more scientific. Our study showed that more than 80% of 245 lactating mothers in Jiangning District of Nanjing city did not reach the Chinese RNI value at 30–50 days and 90–110 days after delivery. This result was similar with the previous survey of 537 lactating mothers in Beijing, Suzhou and Guangzhou. Among them, 86.4% of participants had lower vitamin A intake than the RNI value [7]. However, our previous studies on urban and rural areas in 13 provinces and municipalities showed that more than 90% of Chinese lactating mothers had lower dietary vitamin A intake than the RNI value in 2018 [5]. The reasons for this difference may be the inconsistency of dietary survey methods, the limited dietary sources of vitamin A, the influence of geography, customs and economic conditions, etc.

Previous studies have shown that different forms of vitamin A intervention can improve maternal serum retinol concentration in lactating mothers. For example, a high-dose vitamin A intervention trial in Brazil showed that compared with the control group, the intervention of 200 000 IU of vitamin A on the 20th and 30th day after delivery significantly increased the serum retinol concentration (1.17 ± 0.34 μmol/L vs.

1.02 ± 0.28 μmol/L) and reduced the vitamin A deficiency prevalence (3.2% vs. 16.7%) in lactating mothers at 3 months postpartum [21]. The daily intervention trials so far were about provitamin A, mainly β-carotene. Two studies in Bangladesh and Vietnam found a significant increase in serum retinol concentrations of lactating mothers who received daily high-dose β-carotene rich foods compared to controls [10] [22] [23]. β-carotene can be converted into retinol in vivo, but its absorption and conversion rate are low. A previous study found that daily supplementation of low-dose β-carotene did not improve serum retinol concentration in lactating mothers [24]. Therefore, we used low-dose vitamin A supplementation in the form of retinol, which has high absorption rate and can effectively reduce the safety risk of high-dose vitamin A supplementation. The common form of vitamin A supplement in China is combined with vitamin D, and the dose of vitamin A for adults is 1800 IU. We found that the daily intervention of this dose of vitamin A for 2 months significantly increased the serum retinol concentration of lactating mothers and decreased the marginal vitamin A deficiency rate, which was similar as the results of previous studies mentioned above.

During lactation, vitamin A from dietary sources can be actively transported to the mammary gland through chylomicrons and secreted into breast milk. Vitamin A in breast milk should be maintained an appropriate level to ensure adequate vitamin A supply in the first six months of life for infants who are exclusively breastfed and to prevent possible clinical problems. Vitamin A status in breast milk of participants involved in our study was not ideal, especially at the end of the trial, the main reason is the low dietary vitamin A intake and the concentration of vitamin A in the breast milk decreased over the progress of lactation, which is similar to the results of other studies [25]. At present, the effects of different forms of vitamin A intervention on maternal breast milk retinol concentrations are inconsistent. A number of studies in Bangladesh, Indonesia and Vietnam have shown that a single high-dose vitamin A supplementation (200 000 IU), weekly vitamin A (7000 μg or 4800 μg) supplementation, and daily or weekly supplementation of β-carotene supplementations, β-carotene or β-cryptoxanthin fortified foods, or animal and plant foods rich in vitamin A have increased the concentration of retinol in breast milk [22]. [10] [26] [23] [24]. Consistent with these findings, our study found that 1800 IU of vitamin A intervention per day for 2 months could reduce the decrease of retinol concentration in breast milk of lactating mothers. At the end of the trial, the retinol concentration in breast milk of supplement group was significantly higher than that of control group. However, some studies in Ghanaian [27] and Brazilian [28] have found that vitamin A intervention has no effect on retinol concentration in breast milk. This may be due to the different forms and doses of vitamin A supplements. Both studies were conducted in the form of multivitamins, with a low dose of 800 μg retinol equivalent. The supplement time is also different, one of the trial was from the beginning of pregnancy to the end of labor. Moreover, the baseline maternal serum retinol concentration was at a high level before the start of supplementation, which may also lead to ineffective results.

Surprisingly, we did not find an association between maternal low-dose vitamin A supplementation and the lower risks of infant diseases (febrile illness, respiratory tract infection, diarrhea, and eczema). At present, there are few studies on the effect of maternal vitamin A supplementation on health status of infants. One study assessed the effect of two times of 200 000 IU vitamin A intervention of lactating

mothers on incidence rate of infant diseases, including febrile illness, respiratory tract infection, diarrhea, but not eczema, and the results were consistent with our findings [29]. Although vitamin A has a certain effect of enhancing immunity, the immunity of infants within six months after birth is mainly guaranteed by the mother through breast milk. Colostrum is rich in immune factors, and the prebiotics in breast milk are also conducive to the growth of intestinal probiotics [30] [31] [32]. This may be one of the reasons why vitamin A intervention does not affect the health of infants. In addition, the coexistence of multiple micronutrient deficiencies in breast milk may be another reason. During the 2-month follow-up period, a small number of infants in both the two groups developed f febrile illness, respiratory tract infection, diarrhea, and eczema. Our study found that the dietary intake of water-soluble vitamins (vitamin B1, vitamin B2, vitamin B9 and vitamin C) and minerals (calcium, magnesium and iodine) of lactating mothers were at low levels (data not shown). The content of water-soluble vitamins, iodine and other nutrients in breast milk is closely related to the diet of lactating mothers. Almost all the water-soluble vitamins needed by infants come from breast milk [33] [34]. If the mothers lack these nutrients, the related deficiency symptoms will quickly appear in the infants. Further testing of micronutrients in breast milk is needed to confirm our hypothesis.

In conclusion, supplementation of lactating mothers with daily oral low-dose vitamin A (1800 IU) for 2 months was found to have a positive impact on maternal serum and breast milk vitamin A status, but no effect on infant health status was detectable 2 months after maternal supplementation. Due to ethical constraints, we did not obtain blood from infants to determine their serum retinol concentrations at baseline and 2 months after intervention. However, the results of this study are sufficient to show that daily supplementation of regular dose of vitamin A to lactating mothers is helpful to improve the maternal vitamin A status, and can also play a positive role in vitamin A status of infants through breast milk. This result also provides a certain theoretical basis for dietary guidance for lactating mothers in China, that is, in addition to adding vitamin A rich foods, regular dose of vitamin A supplements are also an optional way for lactating mothers.

5. Conclusions

Daily oral low-dose vitamin A supplementation is helpful to improve the maternal vitamin A status and can also play a positive role in vitamin A status of infants through breast milk.

Abbreviations

BMI, body mass index;

CV, coefficients of variation;

CI, confidence interval;

$\chi 2$, chi square;

IQR, inter-quartile range;

RNI, recommended nutrient intake;

RAE, retinol activity equivalents;

RR, risk ratio;

SD, standard deviation;

UPLC, ultra-high performance liquid chromatography;

VAD, vitamin A deficiency.

Declarations

Ethical Approval and Consent to participate

This study was approved by the ethics committee of Nanjing Medical University and registered at clinicaltrials.gov. Study for vitamins and fatty acids status of breast milk and effects of related supplementation during lactation on the health of mothers and infants: a randomized clinical trial, ChiCTR1800020179.Registered 19 December 2018, http://www.chictr.org.cn/showproj.aspx?proj=33888.

Consent for publication

Not applicable.

Availability of supporting data

The datasets generated and analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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Conflicts of Interest:. The authors declare that they have no competing interest.

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Figures

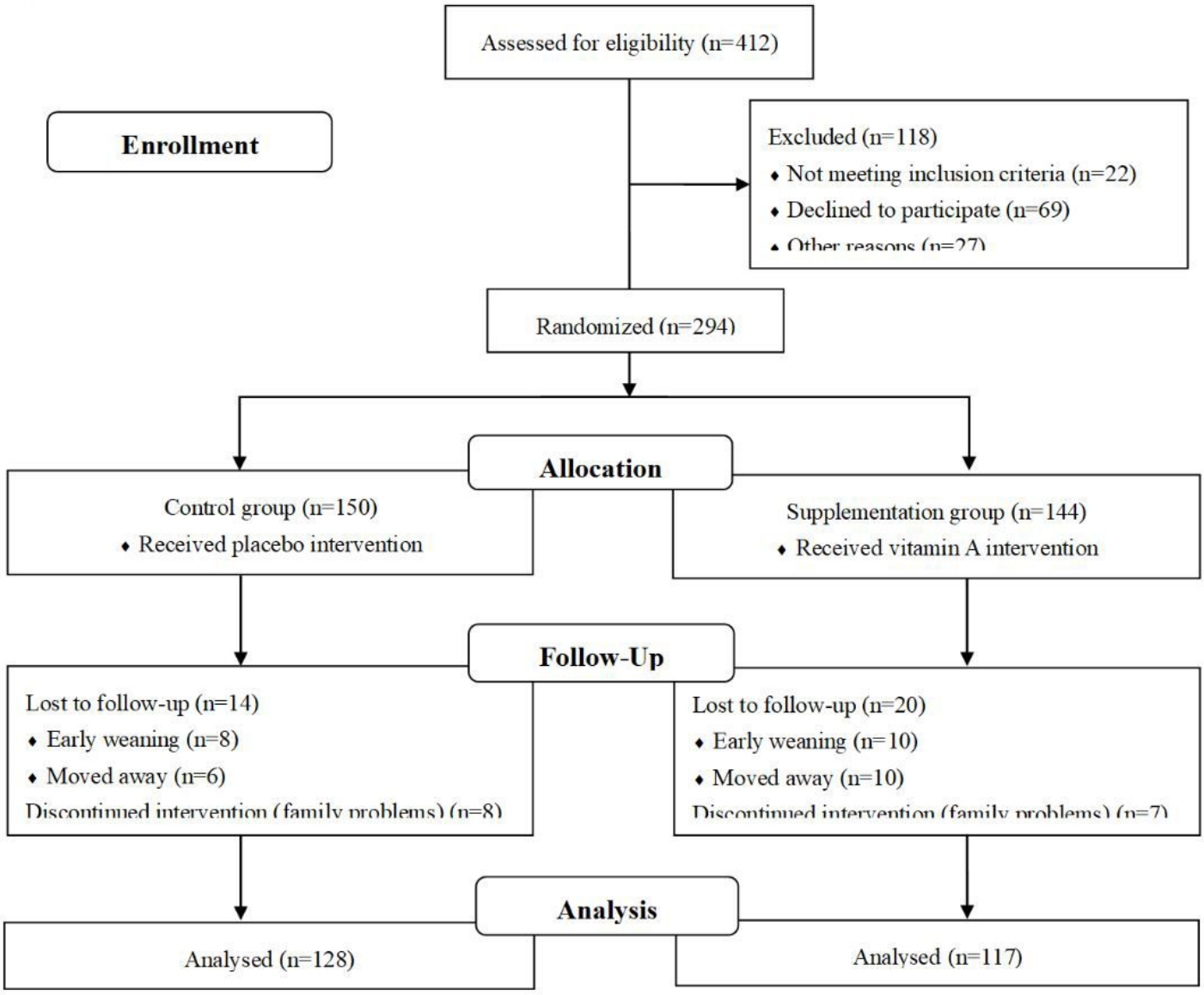

Figure 1

Flow diagram of the final sample for the analysis

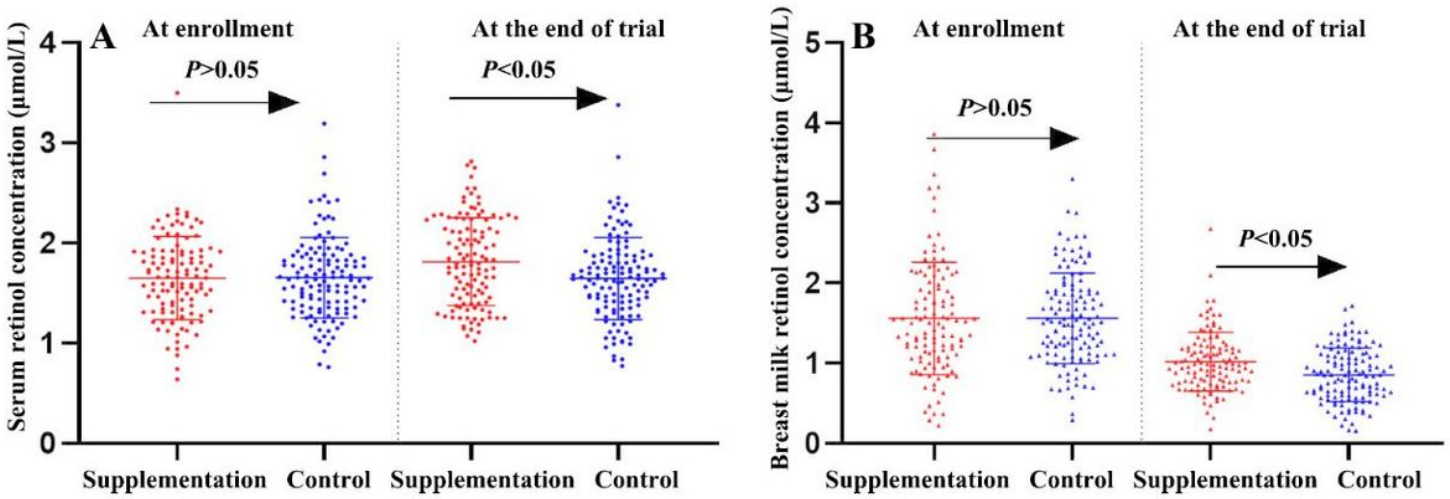

Figure 2

Comparison of retinol concentration in maternal serum (A) and breast milk (B) between supplementation and control groups at enrollment and the end of trial. Values are Means ± SDs. P-values were assessed using student's t test