Original Article

Effects of egg and vitamin A supplementation on hemoglobin, retinol status and physical growth levels of primary and middle school students in Chongqing, China

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Lack of protein and vitamin A influences the growth of student in impoverished mountain areas. The aim of the study was to assess the effects of egg and vitamin A supplementation on hemoglobin, serum retinol and anthropometric indices of 10-18 years old students of a low socioeconomic status. A total number of 288 students from four boarding schools were randomly selected by using cluster sampling method in Chongqing, and they were assigned into supplement group and control group non-randomly. Students in supplement group received a single 200,000 international units vitamin A and 1 egg/day (including weekends) for 6 months. The control group did not receive any supplementation. We measured hemoglobin, serum retinol and height and weight at baseline and after supplementation. The supplementation increased the mean hemoglobin concentration by 7.13 g/L compared with 1.38 g/L in control group ($p<0.001$), the mean serum retinol concentration by 0.31 μmol/L compared with 0.09 μmol/L in the control group ($p=0.005$), the mean height-for-age z score by 0.05 compared with 0.03 in the control group ($p=0.319$), the mean weight-for-age z score by 0.05 compared with -0.12 in the control group ($p<0.001$). Our results revealed that egg and vitamin A supplementation is an effective, convenient, and practical method to improve the levels of hemoglobin, serum retinol and prevent the deterioration of growth in terms of weight for primary and middle school students from outlying poverty-stricken areas. Our intervention did not have a beneficial effect on linear growth.

Key Words: egg, vitamin A, supplementation, effects, students

INTRODUCTION

Nutrition should be a priority at national and sub-national levels because it is central for human, social, and economic development.[1] Poverty is the main underlying cause of malnutrition and its determinants.[2,3] In developing countries, malnutrition with its two constituents, protein-energy malnutrition and micronutrient deficiencies, continues to be a major health burden.[4] China is the biggest developing country in the world with more than 1.3 billion people. Along with the rapid socioeconomic development in the latest 20 years, China has achieved impressive progress towards achieving the Millennium Development Goals (MDGs) and there has been a dramatic reduction in hunger and undernutrition.[1] However, nutritional problems are still widespread among Chinese children, about 10.6%-38.3% of children have anemia and 40% of children have growth retardation in rural areas.[5,6] At the same time, children are prone to a lack of vitamin A, which is mainly from meat products. Dietary survey showed that porridge, steamed bread and potatoes without preformed vitamin A or provitamin A were given to most primary and middle school students in poor areas of western China. One of the major objectives of the National Program of Action for Child Development in China (2001-2010) is to reduce vitamin A deficiency (VAD) and marginal vitamin A deficiency (MVAD).[7]

One of our previous study showed that the occurrence

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of anemia, VAD and MVAD in preschool children in a suburb of Chongqing, China was 26.6%, 7.7% and 34.8 %, respectively.[8] We considered that anemia and VAD are also serious among primary and secondary school students in the poorer area of Chongqing because of their dietary pattern. Our baseline survey found that the prevalence rate of anemia, VAD, MVAD, stunting and wasting were 18.3%, 14.6%, 50.8%, 24.7% and 6.2%, respectively in the studied population.[9] The purpose of this study is to investigate the effects of egg and vitamin A on improving hemoglobin (Hb) and serum retinol status, physical growth of 10-18 years old students in a rural area of Chongqing, China. We hypothesized that the school feeding program was effective in improving the nutrition status of the students in the supplement group. Our program was strongly supported by Chinese government authorities.

MATERIALS AND METHODS

Subject selection

The supplementation program was reviewed and approved by the institutional ethical committee of the Children's Hospital of Chongqing Medical University. We conducted the study in Wuxi County of Chongqing, China from December 2010 to May 2011. Wuxi County is a rural district and a typical mountainous area with low socioeconomic development. As part of the child nutrition project of Wuxi County, we selected four boarding schools which included two primary schools and two middle schools randomly with cluster sampling. The four schools were divided into two groups (the supplement group and the control group) non-randomly, each group included one primary school and one middle school. The supplements were provided for the students in poorer school districts. About 30 students in the supplement group was the minimum sample size to detect an absolute change of 10 g/L of Hb and 0.25 μmol/L of serum retinol after supplementation with 90% power and α=0.05 for a two-sided paired t-test. Considering the attrition over the duration of the study, we selected more than 50 students from each school.

A total of 288 students were recruited. The inclusion criteria for participation were as follows: 1) students of either sex aged 10-18 years; 2) students who had no recent acute or chronic diseases and Hb >60 g; 3) students who were not accepting any supplement; and 4) students whose parents or guardians signed and returned consent (written) to participate in all aspects of the study. However, because of the unexpectedly high dropout rate for involvement in the trial and blood drawing failure, we were supposed to measure the primary outcome including Hb concentration, retinol concentration and anthropometric indices before and after intervention from all of 201 students.

Intervention

The supplement group received 200,000 international units (IU) of vitamin A in capsule formed with retinol acetate (Xiamen Xingsha Pharmaceutical Group Co, Ltd) during December 2010, and then we added one egg to their breakfast everyday, including weekends for 6 months. Supplements provision was recorded and supervised by the head teachers. The control group did not receive any supplement.

Anthropometric measurements

Anthropometric examinations in each school were conducted by the same trained investigators (a total of 4) by following a standardized procedure to eliminate intraexaminer error. Duplicate measurements were performed for all participants. The inter-examiner coefficient of variation of weight and height for each examiner was less than 5%. Weight was recorded using a weighing scale (100- Med, Beijing, China) to the nearest 0.1 kg with subjects in minimum clothing and bare feet. And height was measured in the standard position by the same standing scale (100Med) to the nearest 0.1 cm.

Blood samples and biochemical measurement

Blood samples (about 4 mL) of each subject were collected by venipuncture from the antecubital vein before breakfast. About one-half of blood was drawn into an EDTA anticoagulation tube to measure Hb by the hemiglobincynide (HiCN) method.[10] The inter-assay variation was lower than 5% and the intra-assay variation was lower than 10%. The remaining blood was centrifuged at 3500 rotations per minute×10 min at 4°C and then separated immediately. The centrifuged serum samples were transported to the laboratory away from light and stored at -20 °C. The concentration of serum retinol was determined by using HPLC (Waters 2487UV detector USA).[11] Retinol was extracted with hexane after deproteinization with ethanol containing retinyl acetate as the external standard and evaporated to dryness with nitrogen gas. The residual materials were dissolved in 0.1 mL of mobile phase which was a methanol: DH_2O mixture (97:3). A portion (20 μL) of the sample was injected into a column (3.9×150 mm; Symmetry Shield RP_{18} Waters Breeze, Milford, MA, USA) installed with a high-performance liquid chromatographic apparatus (Waters 1525 Binary HPLC Pump, Waters Breeze). All procedures were performed in a dark room to protect the serum from light. The concentration of retinol was determined with a spectrophotometer (Waters 2487 Dual 1 Absorbance Detector, Waters Breeze) at 315 nm. Duplicate analyses were performed on one-tenth of the samples and the estimated variability was 0.02 μmol/L. The experienced examiners in the Pediatric Laboratory of Chongqing Medical University measured all biochemical indices.

Definition of outcomes

Z-scores were calculated according to the US CDC child growth reference (2000) by Epi info 2002 for height-for-age (HAZ) and weight-for-age (WAZ).[12] A cut-off of less than minus two standard deviations (-2 SD) was used to define stunting (HAZ, -2), and underweight (WAZ, -2).[13] Prevalence of anemia was determined according to the WHO criteria,[14] 1) Hb <115 g/L for 5-11-year-olds; 2) Hb <120 g/L for 12-14-year-olds and girls more than 15 years; 3) Hb <130 g/L for boys age >15 years. According to the WHO criteria,[15] a serum retinol concentration <0.7 μmol/L was classified as VAD and values between 0.70 and 1.05μmol/L were defined as marginal vitamin A deficiency (MVAD).

Statistical analysis

The data entry forms were designed with Microsoft Office Excel with range and consistency checks incorporated. Statistical analysis was performed with SPSS software version 19. Continuous variables were presented as mean±SE while categorical variables were presented as percentage rate. Paired-t tests were used to compare paired data, the differences in change between two groups were estimated with ANOVA and the percentage rates were assessed with chi-square test. A value of $p<0.05$ was considered statistically significant.

RESULTS

The characteristics of the participants

Two hundred and eighty-eight primary and middle school students were recruited. Among the children, 35 were excluded (3 for Hb level <60 g/L, 21 for age or sex information is not complete, 11 for recent acute disease), and 253 children met the study inclusion criteria (101 for supplement group, 152 for control group). About 20% (52 of 253) dropped out during the course of the study. The dropout numbers were 25 (4 for blood drawing failure and 21 for moving during the trial) for the supplement group, 27 (2 for blood drawing failure and 25 for moving during the trial) for the control group. Thus, primary outcome measurements were obtained from 201 school children (76 in the supplement group, 125 in the control group).

The age (mean±SE) and ratio of boys to girls were 12.4±0.20 years and 40 to 36 in supplement group, 12.2±0.15 years and 61 to 64 in control group. Differences between groups in terms of nutritional characteristics and anthropometric measurements at the beginning of the study showed the need for adjusted comparisons (Table 1).

Effect of intervention on biochemical parameters

The concentrations of Hb, retinol at baseline and at the end of the study, as well as the change over 6 months of intervention are summarized in Table 2. The concentration of Hb increased significantly ($p<0.001$) within supplement group over the 6 months. The concentration of retinol increased significantly in the supplement group ($p<0.001$) and in the control group ($p=0.003$) over the intervention period. The increase in retinol concentration in the control group was less significant than in supplement group ($p=0.005$; Table 3).

Effect of intervention on physical measurements

The anthropometric indices (expressed as z-scores) at baseline, final measures and changes from baseline over the intervention period are also presented in Table 2. The increases in mean height-for-age during the 6 months in each group were not big enough to be statistically significant (Tables 2 and 3). The weight-for-age decreased significantly in the control group ($p=0.001$; Table 2) but not in the supplement group ($p=0.311$; Table 2).

To further explore the data, we calculated the rates. The rates of anemia, VAD, MVAD in the supplement group decreased significantly compared with that in the control group (Table 4). The decrease in rates of stunting in each group was not significant. The rates of underweight in the control group increased but was not statistical significance ($p=0.284$). After the study we found that egg and vitamin A supplementation is an effective method to improve the levels of hemoglobin, serum retinol and prevent the deterioration of weight for the subjects. But our intervention did not have a beneficial effect on linear growth.

DISCUSSION

Malnutrition is still highly prevalent in rural areas of China because the typical diet consists of mainly cereals and lacks the optimal diversity and quality to meet the nutrient needs of most people.[16] Adolescence in the life cycle is characterized by rapid growth and maturation. At this stage of development, the majority of adolescents also actively engage in sports and recreational activities. Hence, sufficient energy and nutrient intake from balanced diets and are necessary for optimal growth. Lack of nutrition source may affect nutritional status of the students in economically challenging areas. Common nutritional problems are anemia, VAD, stunting, wasting and etc. This study was conducted in rural schools of a low socioeconomic background in a developing country to investigate the effect of egg and vitamin A supplementation on school children. We chose boarding schools with cluster sampling to ensure that subjects ate school meals during the period of 6 months. Ethical consideration precluded randomization therefore there were differences between two groups at baseline, we adjusted baseline data by using Multi-way ANOVA to analyze the differences of changes between two groups.

The supplements selection

Protein (especially high-quality protein) is very important

Table 1. The characteristics of the participants[†]

Characteristic	Supplement group (n=76)	Control group (n=125)
Age, year	12.4±0.20	12.2±0.15
Sex, M,F	40,36	61,64
Hemoglobin, g/L	130±0.95	129±1.04
Serum retinol, μ mol/L	0.90±0.03	1.00±0.03
Height, cm	148±0.96	142±0.83
Weight, kg	39.3±0.95	34.8±0.66
z-scores		
HAZ	-0.83±0.13	-1.42±0.09
WAZ	-0.92±0.14	-1.52±0.09

[†]Values are mean±SE.
HAZ: z-score for height; WAZ: z-score for weight.

Table 2. Biochemical parameters and anthropometric indices at baseline, final measure, and changes due to the intervention. †

Parameters	Supplement group (n=76)					Control group (n=125)				
	Baseline	Final	Change	95%CI	p‡	Baseline	Final	Change	95%CI	p‡
Hb, g/L	130±0.95	137±1.09	7.13±0.84	5.46-8.80	<0.001	129±1.04	130±1.09	1.38±1.27	-1.13-3.90	0.278
Retinol, µmol/L	0.90±0.03	1.21±0.03	0.31±0.03	0.25-0.37	<0.001	1.00±0.03	1.09±0.02	0.09±0.03	0.03-0.15	0.003
HAZ	-0.92±0.14	-0.86±0.13	0.05±0.07	-0.09-0.19	0.477	-1.42±0.09	-1.39±0.08	0.03±0.04	-0.05-0.11	0.497
WAZ	-0.83±0.13	-0.78±0.12	0.05±0.05	-0.05-0.16	0.311	-1.52±0.09	-1.64±0.08	-0.12±0.04	-0.19- -0.05	0.001

†Values are means±SE.
‡Paired-t test.
HAZ: z-score for height; WAZ: z-score for weight.

Table 3. Differences between supplement group and control group in mean change in hemoglobin, serum retinol, HAZ, WAZ from baseline after 6-months intervention†

Parameters	Supplement group (n=76)	Control group(n=125)	p
ΔHb, g/L	7.13±0.84	1.38±1.27	<0.001
Δretinol, µmol/L	0.31±0.03	0.09±0.03	0.005
ΔHAZ	0.05±0.07	0.03±0.04	0.319
ΔWAZ	0.05±0.05	-0.12±0.04	<0.001

†Mean changes ±SE (follow-up minus baseline). Values are adjusted for baseline hemoglobin, retinol, height and weight with ANOVA.
HAZ: z-score for height; WAZ: z-score for weight.

Table 4. Comparison of prevalence of anemia, VAD, MVAD, stunting and underweight from baseline to 6-month follow-up in the supplement group and the control group†

Parameters	Supplement group (n=76)					Control group (n=125)				
	Baseline	Final	Change	χ^2	p	Baseline	Final	Change	χ^2	p
Anemia	12 (15.8)	3 (3.9)	-9 (-11.9)	7.87	0.005	12 (8.8)	20 (16.0)	8 (7.2)	2.29	0.130
VAD	13 (17.1)	1 (1.3)	-12 (-15.8)	11.3	0.001	13 (10.4)	8 (6.4)	-5 (-4.0)	1.30	0.254
MVAD	41 (53.9)	16 (21.1)	-25 (-32.8)	37.7	<0.001	65 (52.0)	56 (44.8)	-9 (-7.2)	1.30	0.255
Stunting	14 (18.4)	12 (15.8)	-2 (-2.6)	0.186	0.667	37 (29.6)	35 (28.0)	-2 (-1.6)	0.078	0.780
Underweight	11 (14.5)	9 (11.8)	-2 (-2.7)	0.230	0.631	38 (30.4)	46 (36.8)	8 (6.4)	1.147	0.284

†Values are n (%)

for students in primary and secondary school, the report of a joint FAO/WHO/UNU expert consultation suggest that protein requirement for adolescent is about 0.9 g/kg/d.[17] Egg is the best general source of protein while every 100 g egg contains 13.3 g protein, the biological value and the digestibility of egg protein is 94% and 97%. [17]

Vitamin A (VA) plays an important role in cell differentiation and maturation, VAD may cause exophthalmia and lead to blindness, anemia, limit growth, weaken innate and acquired host defenses, exacerbate infection and increase the risk of death.[18] There are three major strategies to combat VAD, which are food fortification, supplementation with vitamin A pharmaceutical preparations and food diversification. Each of these three strategies has disadvantages and limitations. Many agree that food diversification offers the best long-term approach that is likely to be sustainable.[19] But often it requires either major change in agricultural production, including home gardens, or in higher incomes for the poor, allied with nutrition education. Providing VA-fortified food is a direct and effective method to improve the VA nutritional status.[20-22] Many large-scale intervention trials showed that high dose of vitamin A capsule supplementation reduced the mortality rate of 23% to 30% of children, proved that this was a very cost-effective intervention in low-income countries.[23,24] WHO recommends the doses of vitamin A supplements as 100,000 IU at the age of 6-11 months and 200,000 IU every 3-6 months at the age of ≥12 months.[25] In this study, we chose 200,000IU VA capsule and egg instead of multiple micronutrients package to prevent the malnutrition with consideration of economic and convenient factors, and the results shows that it was efficacious.

Effect of intervention on biochemical parameters

The results of our study revealed supplementation with egg and vitamin A for 6 months had good effects on hemoglobin and serum retinol levels. After intervention, the concentration of hemoglobin and serum retinol was markedly increased in the supplement group compared with that in the control group. Anemia, VAD and MVAD rates decreased by around 11.9%, 15.8%, and 32.8% in supplement group. The effect on hemoglobin in the study may not only be due to the protein provided by egg but also the function of retinoid. Vitamin A has been shown to improve hemoglobin concentrations and increase the efficacy of iron supplementation.[26] The mechanisms are not fully understood, but are suggested to operate through effects on transferrin receptors affecting the mobilisation of iron stores, increasing iron absorption,[27] stimulating erythroid precursors in the bone marrow,[28] and reducing susceptibility to infections.

Effect of intervention on physical measurements

The results showed that after the 6 months of intervention, weight-for-age decreased significantly in the control group but there was no significant change in the supplement group. Therefore the short time supplement only prevented the deterioration of weight-for-age but did not improve it. Regretfully, our intervention did not change height-for-age in each group.

Undoubtedly protein can promote adolescent growth and development, and the negative effect of VAD on growth and development is almost unquestionable. A large number of animal experiments have clearly demonstrated this point.[18] One human study also found that stunting children with VAD experience reduced growth hormone secretion at night.[29] But contrary to this, some other studies have suggested that mass-dose vitamin A had no effect on children's anthropometry, growth velocity, z-scores, or body composition compared with the placebo-controlled group.[30-32]

Our result of height may be because of the following reasons. Firstly, weight-for-age mainly reflects recent nutritional status while height-for-age is a long-term consequence of earlier malnutrition and mainly effected by genetic factors. Karlberg analyzed the height of 3650 Swedish infants from birth to 18 years, indicated that parental height affected on infant birth length seriously and birth length was significantly associated with final height.[33] A Cochrane review of school feeding strategies in older children suggests that the effect could lead to an increase in body-mass index rather than a substantial effect on stunting.[34] In the past most reports and assessments of nutrition programs have focused on weight gain rather than linear growth. In general, stunting and linear-growth retardation are regarded as difficult to change, we need to focus attention on interventions in pregnancy and in young children, especially those under 24 months of age.[35] Secondly, due to the financial constraints we did not diversify the students' diet and thirdly, the nutrition supplement lasted a short period of time, the intervention was not clearly effective on physical growth of school-aged children.

There are some limitations in the current study. Because of the limited design of the original protocol, we did not supply other micronutrient simultaneously and evaluate some other confounding factors, such as iron, serum zinc, or unsaturated fatty acid, CRP etc, which are capable of affecting anemia and physical growth. The dropout rate in our study was unexpectedly high (20%), mainly because a portion of the enrolled school children entered higher schools. Increasing the variety of school foods and early nutritional interventions on pregnant women, and infants will be important aspects in future studies.

In summary, our results indicate that egg and vitamin A have useful roles on hemoglobin, serum retinol, and weight-for-age. School feeding, can be defined as the provision of food to children through schools to improve their attendance, achievement, growth, and other health outcomes,[34,36] has a rather short history in China, but has made great achievements during the late few years. In long term, the first and most important task is to promote the acts of registered school feeding system, and assist the government to make official policies to support the system.

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AUTHOR DISCLOSURES

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补充鸡蛋和维生素 A 改善中国重庆中小学生血红蛋白、血清视黄醇浓度和体格生长水平的效果

蛋白质和维生素 A 缺乏严重影响贫困山区学生的生长发育。本次研究主要探讨补充鸡蛋和维生素 A，改善贫困地区 10-18 岁学生之血红蛋白、血清视黄醇浓度及体格生长的效果。采用随机整群抽样方式，选取重庆 4 所寄宿制学校，总共 288 名学生纳入研究，将其非随机地分为补充组和对照组。补充组学生一次性口服 200,000 单位维生素 A 胶丸，并每天补充一个鸡蛋(包括周末)持续 6 个月；对照组不接受任何营养补充。在补充前和补充后，分别测定受试学生的血红蛋白、血清视黄醇浓度、身高和体重。结果显示，补充组血红蛋白浓度增加 7.13 g/L，明显优于对照组的 1.38 g/L (p<0.001)。补充组血清视黄醇增加 0.31 μmol/L，也明显高于对照组的 0.09 μmol/L (p=0.005)。另外补充组身高 z 评分增加 0.05，对照组增加 0.03，两者无统计学差异(p=0.319)；但补充组体重 z 评分增加 0.05，明显优于对照组的-0.12 (p<0.001)。総结而言，补充鸡蛋和维生素 A 是一种用于改善偏遠贫困地区中小学生血红蛋白、血清视黄醇及阻止体重减轻的高效、方便、实际易操作的营养干预方式；但本次营养补充并没有对身高产生有益影响。

关键字：鸡蛋、维生素 A、补充、效果、学生