Vitamin A Supplementation of Women Postpartum and of Their Infants at Immunization Alters Breast Milk Retinol and Infant Vitamin A Status[1]

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ABSTRACT Vitamin A supplementation of lactating mothers and of infants at the time of diphtheria-pertussis-tetanus (DPT) and oral polio vaccine (OPV) immunizations have both been suggested as measures to prevent deficiency among infants. This multicenter randomized, double-blind, placebo-controlled trial was conducted in Ghana, India and Peru to determine the effect of maternal vitamin A supplementation on breast milk retinol and of maternal and infant supplementation on infant vitamin A status. Mothers in the intervention group received 60 mg vitamin A (as retinol palmitate) at 18–42 d postpartum; their infants were given 7.5 mg three times, i.e., at 6, 10 and 14 wk of age with DPT and OPV immunizations. Mothers and infants in the comparison group received a placebo. Maternal supplementation resulted in higher breast milk retinol at 2 mo postpartum [difference in means 7.1, 95% confidence interval (CI), 3.4, 10.8 nmol/g fat] and lower proportion of mothers with breast milk retinol $\leq$ 28 nmol/g fat (15.2 vs. 26.6%, 95% CI of difference −16.6, −4.1%). At 6 and 9 mo, maternal supplementation did not affect breast milk retinol or the proportion of mothers with low breast milk retinol. Vitamin A supplementation of the mothers and their infants reduced the proportion of infants with serum retinol $\leq$ 0.7 μmol/L (30.4 vs. 37%, 95% CI of difference −13.7, 0.6%) and that with low vitamin A stores as indicated by the modified relative dose response (MRDR) $>$ 0.06 (44.2 vs. 52.9%, 95% CI of difference −16.6, −0.9%) at 6 mo. Supplementation had no effect at 9 mo. The beneficial effect of supplementation on breast milk retinol and infants' vitamin A status varied by site. It was greatest in India followed by Ghana and Peru. At the doses used, maternal supplementation improved breast milk retinol status at 2 mo ($P < 0.001$) and maternal and infant supplementation modestly increased ($P = 0.03$) infant vitamin A status at 6 mo of age. Additional strategies to improve vitamin A status of 6- to 9-mo-old infants must be considered. J. Nutr. 132: 3243–3248, 2002.

KEY WORDS: • *human infants* • *vitamin A supplementation* • *breast milk retinol* • *serum retinol*

Around 1990, clinical or subclinical vitamin A deficiency existed in an estimated 118 countries in the world and affected >250 million children < 5 y old (1). Three independent meta-analyses of studies of periodic high dose vitamin A supplementation to children aged 6 mo to 5 y have reported a 20–30% reduction in all-cause mortality (2–4). Many developing countries have vitamin A supplementation programs for children at this age. Vitamin A supplementations to lactating mothers to improve infant's vitamin A status through increased breast milk retinol, and to infants at 6, 10 and 14 wk with vaccines under the Expanded Program for Immunization (EPI)[4] have been suggested as measures to prevent deficiency in the first 6–9 mo of life (5).

A randomized, double-blind, placebo-controlled multicenter trial was coordinated by the WHO to determine the benefits and safety of maternal and immunization-linked vitamin A supplementation in Ghana, India and Peru. The intervention was 60 mg vitamin A to mothers within 6 wk of delivery and 7.5 mg to their infants at 6, 10 and 14 wk of age with diphtheria-pertussis-tetanus (DPT) and oral polio vaccine (OPV) immunizations. The findings of this trial that relate to safety and the effect on morbidity, mortality and vitamin A status of infants have already been published (6). The publication reported only overall results related to the effect on infant vitamin A status. In this paper, we now present the overall and site-specific data on the effect of

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[4] Abbreviations used: CI, confidence interval; DPT, diphtheria-pertussis-tetanus; EPI, Expanded Program on Immunization; MRDR, modified relative dose response; OPV, oral polio vaccine.

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maternal supplementation on breast milk retinol and site-specific data on the effect of combined maternal and infant supplementation on infant vitamin A status.

SUBJECTS AND METHODS

A total of 9424 mother-infant pairs were enrolled in the trial, 18–28 d postpartum in India and Peru and 21–42 d after delivery in Ghana. They were randomly assigned to receive vitamin A or placebo. The mothers were administered a single dose of 60 mg vitamin A or placebo at enrollment.[5] Infants of mothers in the vitamin A group received 7.5 mg vitamin A at each of the EPI immunizations at 6, 10 and 14 wk of age in Ghana and India and at 2, 3 and 4 mo of age in Peru. Infants of mothers in the placebo group received a placebo at each immunization. At 9 mo of age, all infants were administered vitamin A, 7.5 mg to the vitamin A group and 30 mg to those in the placebo group, with the measles immunization, according to the national program policies. Vitamin A was provided as retinol palmitate with minute amounts of vitamin E; the placebo was soybean oil.

At each study site, ~25% of the 4000 mother-infant pairs that were part the main study were randomly selected for the biochemical substudy; at least 80% of these gave breast milk and blood samples. A breast milk sample was collected from all of these mothers at enrollment. A second breast milk sample was obtained at 2, 6 or 9 mo postpartum in randomly selected subgroups. Blood samples were obtained at 6 wk from infants whose mothers were selected to provide a breast milk sample at 2 mo. Blood samples of infants whose mothers were selected for a second breast milk sample at 6 and 9 mo were obtained at the same time. This strategy ensured that each infant in the biochemical substudy provided only one blood sample and the mother provided two breast milk samples, one at enrollment and the second at the time when the blood sample was collected from the infant.

Informed written consent was obtained from parent(s) before enrollment. The study was approved by the appropriate ethics review committees of all participating institutions and the WHO ethics review board.

Sample size. The sample size was calculated to allow a site-specific analysis. In the vitamin A and placebo groups in Ghana, India and Peru, ~100 infants from each group were selected for breast milk and infant blood sampling at 6 wk, 6 mo, 9 mo and 1 y. This sample size was adequate to detect a 0.5 SD difference in mean serum retinol and mean breast milk retinol and a 40% reduction in the proportion of infants with modified relative dose response (MRDR) > 0.06 with 95% confidence and 80% power (7).

Laboratory methods. Breast milk samples (10 mL) were collected in amber-colored glass bottles from either one of the breasts using a breast pump. Because the infants were breast-fed on demand, breast milk samples were collected independently of the time since the previous feed, usually between 0900 and 1200 h. Three capillary tubes were filled with breast milk; creamatocrit, which was used to estimate the fat content of breast milk, was measured as described (8). Breast milk samples were stored at −20°C or below. The temperature of the freezer was checked twice daily.

Venous blood samples in India and Peru, and capillary blood samples in Ghana were collected from infants to perform serum retinol and MRDR assays. An analog of vitamin A, didehydroretinol, in a dose of 150 μg was administered to the infant on the morning of the day the blood sample was drawn. After 5 h, a blood sample was drawn into a glass tube covered with aluminum foil. The blood was allowed to clot for 20 min and serum separated by centrifugation at 10,000 × *g* for 5 min. The sera were stored at −20°C in polypropylene tubes covered with aluminum foil to protect from light.

Biochemical analyses using the same standardized methodology were conducted independently at each of the three sites (9). Breast milk samples were homogenized to 200 μL of milk, 100 μL of internal standard (didehydroretinol acetate), 160 μL of a 30% solution of potassium hydroxide in alcohol and 500 μL of ethanol was added. The tubes were capped and the mixture saponified for 1 h at 45°C with periodic mixing. *n*-Hexane (1 mL) was added, vortexed for 30 s and centrifuged at 2500 × *g* for 5 min. Vitamin A was extracted into the hexane layer and the hexane evaporated under nitrogen. The extracted vitamin A was redissolved in mobile phase (90% methanol, 10% water), and a 25 μL sample was injected into the HPLC (Waters, Milford, MA).

The serum (100–500 μL) was first treated with an equal volume of alcohol. Retinyl acetate in methanol was added as an internal standard and vitamin A was extracted twice with an equal volume of hexane. The combined layers were pooled and evaporated under nitrogen. The sample was resuspended in a known volume of mobile phase and injected into the HPLC column. Retinol and didehydroretinol in serum and breast milk were measured by HPLC on reversed-phase C-18 columns with a 90% methanol/10% water mobile phase. The detection wavelengths were 325 nm for breast milk retinol and 350 nm for didehydroretinol. Peak areas were measured by an automatic integrator and didehydroretinol and retinol quantitated against authentic standards.

Internal quality control was maintained by analysis of three aliquots of a reference pool of serum or breast milk samples in each sample lot. The two pools had assigned values set against standard reference material procured from National Institute of Standards and Technology, Gaithersburg, MD. The analysis of a lot was repeated if the intralot variation exceeded 5%. For external quality control, each of the three laboratories took part in a standardization program of the U.S. National Institute of Standards and Technology throughout the study. Of nine rounds analyzed (three times a year) in each of the three laboratories (27 analyses), all but one were within 2 SD of reference values.

Data analysis. Simple comparisons of means by treatment group were used to determine the effect of supplementation on serum and breast milk retinol concentrations. Because the breast was not completely emptied and only a casual sample of breast milk was taken, breast milk retinol was standardized for the fat in the sample. Creamatocrit was related to the fat content measured by extraction on the basis of measurements made on 25 breast milk samples measured by both methods (linear regression analysis: $r = 0.95$, $P < 0.0001$). Using the linear equation obtained, the mean crematocrit of each breast milk sample was converted to fat concentration (g/L). Breast milk retinol concentrations are reported relative to milk fat (nmol/g).

According to the criteria established by the WHO, values ≤ 28 nmol/g fat indicate low breast milk vitamin A concentration and values ≤ 0.7 μmol/L indicate low serum retinol (9). The didehydroretinol to retinol the ratio (MRDR) was used as an indicator of vitamin A stores in the infants. The usual cut-off value of >0.06 for identifying children with low vitamin A stores was used for infants in this study (10–12). Because experience with the use of MRDR in infancy is limited and >50% infants had MRDR > 0.06 at 6 mo of age, we also used >0.09 and >0.12 cut-off values to achieve greater specificity in identifying infants of low vitamin A status (13).

The analysis was performed using SPSS for Windows (version 8.0, Chicago, IL) software. Student's *t* test for continuous data and χ^2 test for qualitative variables were used to test the study hypotheses. The difference in means and proportions between vitamin A and control groups and their 95% confidence intervals (CI) were calculated (14). Values in the text are means ± SD or mean differences and 95% CI.

RESULTS

At enrollment, there were no differences in age, sex, weight, breast-feeding status, maternal age, night blindness during pregnancy, parental education, family size and birth order in the infants in the vitamin A and placebo groups, overall and within each site. The baseline breast milk retinol concentrations also did not differ between subjects who were randomly assigned to vitamin A and placebo groups, overall and within each site (**Table 1**).

[5] Conversion factors: 1 mg vitamin A = 1000 μg retinol equivalents (RE) = 3333 international units (IU).

TABLE 1

Baseline characteristics of enrolled subjects in vitamin A and control groups[1]

Characteristic	Overall		Ghana		India		Peru	
	Vitamin A $n = 1491$	Placebo $n = 1499$	Vitamin A $n = 539$	Placebo $n = 534$	Vitamin A $n = 460$	Placebo $n = 464$	Vitamin A $n = 492$	Placebo $n = 501$
Infants								
Age at enrollment, *d*	23.4 ± 3.0	23.4 ± 2.9	24.5 ± 2.9	24.5 ± 2.9	23.1 ± 2.6	23.0 ± 2.6	22.5 ± 3.2	22.6 ± 3.1
Males, *n (%)*	792 (53)	752 (50)	264 (49)	254 (48)	244 (53)	238 (51)	284 (58)	260 (52)
Breast-fed, *n (%)*	1489 (99.9)	1494 (99.7)	539 (100)	533 (99.8)	458 (99.6)	461 (99.4)	492 (100)	500 (99.8)
Infants weight at enrollment, *kg*	3.6 ± 0.6	3.6 ± 0.6	3.7 ± 0.5	3.6 ± 0.6	3.2 ± 0.6	3.2 ± 0.5	3.9 ± 0.5	3.9 ± 0.5
Birth order, *n*	3.8 ± 1.8	3.8 ± 1.8	4.2 ± 2.0	4.5 ± 2.1	3.4 ± 1.4	3.5 ± 1.3	3.7 ± 1.7	3.5 ± 1.6
Mothers								
Night blindness during pregnancy, *n (%)*	62 (4)	65 (4)	5 (1)	4 (1)	8 (2)	11 (2)	49 (10)	50 (10)
Age of mother, *y*	25.4 ± 5.6	25 ± 5.4	26 ± 6.3	26.2 ± 6.6	24.2 ± 4.1	24.2 ± 4.0	26.2 ± 6.4	25.5 ± 6.1
Mothers' years of education, *n*	5 ± 4.5	4.9 ± 4.6	3 ± 4	2.5 ± 3.8	4.1 ± 4.5	4.0 ± 4.5	8.0 ± 3.3	8.2 ± 3.2
Breast milk retinol, *nmol/g fat*	52.2 ± 27.4	51.6 ± 26.4	60.9 ± 26.5	58.8 ± 24.2	37.5 ± 23.1	38.7 ± 27.4	56.4 ± 26.4	55.7 ± 23.2

[1] All values are means ± SD or *n* (%).

The results are presented overall and by site. Site-specific results are presented because the mother-infant pairs in the study were from rural (Ghana), periurban (Peru) and urban slum (India) populations from three different continents and differed in several baseline characteristics. The median years of schooling of mothers were highest in Peru, followed by India and Ghana. Indian infants were the most malnourished at enrollment, with a mean weight ~0.4 kg lower than Ghanaian and 0.7 kg lower than Peruvian infants. Further, the mean breast milk retinol among controls at baseline was lowest in India (38.7 ± 27.4 nmol/g fat), followed by Peru (55.7 ± 23.2 nmol/g fat) and Ghana (58.8 ± 24.2 nmol/g fat). The proportion of infants in the placebo group at 6 wk in the three sites with serum retinol ≤ 0.7 μmol/L was also highest in India (81.1%), followed by Ghana (60.2%) and Peru (45.1%).

Effect of maternal supplementation on breast milk retinol

Overall analysis. At 2 mo postpartum, vitamin A supplementation improved breast milk vitamin A (**Table 2**). Breast milk retinol was higher (difference in means, 7.1 nmol/g fat, 95% CI 3.4, 10.8) and the proportion of mothers with breast milk retinol ≤ 28 nmol/g fat was lower (15.2 vs. 26.6%, 95% CI of difference −16.6, −4.1%) in the vitamin A group.

At 6 and 9 mo postpartum, mean breast milk retinol concentrations and the proportion of mothers with low breast milk retinol did not differ between the two groups (Table 2).

Site-specific analysis. Analyzing by site, a beneficial effect of maternal supplementation was seen in India and Peru at 2 mo postpartum. The benefit was of a greater magnitude in India both in the difference in breast milk retinol (10.3 nmol/g fat, 95% CI 4.6, 16) and the difference in proportion of mothers with breast milk retinol ≤ 28 nmol/g fat (−22.2%, 95% CI −36.5, −7.9%) between vitamin A and placebo groups (Table 2).

At 6 mo postpartum, the breast milk concentration tended to be higher in the vitamin A-supplemented group in India ($P = 0.05$) (difference in means, 4 nmol/g fat, 95% CI −0.004, 8). There was no effect at 6 mo of supplementation on breast milk retinol status in Ghana or Peru (Table 2). Maternal supplementation did not affect breast milk retinol concentration or the proportion of women with low breast milk retinol in any of the sites at 9 mo postpartum (Table 2).

Effect of maternal and EPI-linked supplementation on vitamin A status of infants

Overall analysis. At 6 mo, serum retinol was not greater in the vitamin A-supplemented group but there was a trend ($P = 0.07$) for a lower proportion of infants with serum retinol ≤ 0.7 μmol/L in this group (30.4 vs. 37%, 95% CI of difference, −13.7, 0.6%). The proportion of infants with a MRDR > 0.06 (44.2 vs. 52.9%, 95% CI of difference, −16.6, −0.9%) was lower in the vitamin A-supplemented group, indicating better vitamin A stores after supplementation. The reduction in the proportion of infants with low stores was greater when a higher MRDR cut-off value was used (**Table 3**).

At 9 mo, the beneficial effects on serum retinol and MRDR status were no longer apparent except on the proportion of infants with MRDR > 0.09 (difference in proportions, −6.2%, 95% CI −12.5, −0.02%).

Site-specific analysis. The overall benefit on infant vitamin A status at 6 mo was due largely to effects in India and to a lesser extent, Ghana. For instance, supplementation increased serum retinol in India and decreased the proportion of infants with low serum retinol in Ghana. The proportion of infants with MRDR > 0.06 was also lower due to vitamin A only in Indian infants (62.9 vs. 76.5%, 95% CI of difference, −26.4, −0.9%) (Table 3).

At 9 mo, vitamin A stores were higher in the supplemented infants only in India. The difference in proportions of infants with MRDR > 0.06 was −4.7% (95% CI −18.9, 9.4%), for > 0.09, −18.9% (95% CI −32.1, −5.7%) and for the > 0.12 cut-off value, it was −12.6% (95% CI −24, −1.2%) in favor of the supplemented group. An effect of supplementation on serum retinol was not observed in 9-mo-old infants in any of the study sites (Table 3).

TABLE

Overall and site-specific effect of maternal vitamin A supplementation given as 60 mg retinol palmitate

	Overall			Ghana		
Indicator	Vitamin A	Placebo	Difference (95% CI)	Vitamin A	Placebo	Difference (95% CI)
Infant age 2 mo	*n* = 322	*n* = 309		*n* = 123	*n* = 124	
Breast milk retinol, *nmol/g fat*	49.8 ± 24.6	42.7 ± 22.1	7.1 (3.4, 10.8)*	56.4 ± 22.8	50.6 ± 21.0	5.8 (−2.8, 5.8)
Breast milk retinol ≤28 *nmol/g fat*	49 (15.2)	79 (25.6)	−10.4 (−16.6, −4.1)*	8 (6.5)	16 (12.9)	−6.4 (−13.7, 0.9)
Infant age 6 mo	*n* = 340	*n* = 334		*n* = 121	*n* = 114	
Breast milk retinol, *nmol/g fat*	42.9 ± 21.6	41.8 ± 25.8	1.1 (−2.5, 4.7)	52.8 ± 21.8	50.6 ± 22.9	2.2 (−3.5, 8)
Breast milk retinol ≤28 *nmol/g fat*	91 (26.8)	109 (32.6)	−5.8 (−12.8, 1)	14 (11.6)	15 (13.2)	−1.6 (−10.8, 6.8)
Infant age 9 mo	*n* = 276	*n* = 294		*n* = 94	*n* = 78	
Breast milk retinol, *nmol/g fat*	43.6 ± 22.4	45.2 ± 27.9	−1.6 (−5.8, 2.5)	49.7 ± 19.4	54.5 ± 25.4	−4.8 (−11.7, 2.1)
Breast milk retinol ≤28 *nmol/g fat*	76 (27.5)	86 (29.2)	−1.7 (−9.1, 5.7)	12 (12.8)	8 (10.3)	2.5 (−7, 12)

[1] All values are means ± SD or *n* (%); * significant difference, $P < 0.05$. CI, confidence interval.

DISCUSSION

Maternal supplementation at the dose used in the current study improved breast milk retinol at 2 mo postpartum but the effect was not sustained to 6 or 9 mo. Previous studies from Bangladesh with the same dose reported a significant effect on breast milk retinol concentration up to 6 mo postpartum (13,15). There is some evidence that a higher dose might give a more extended benefit. Studies that used a ≥ 90-mg dose reported improved breast milk retinol concentrations at 6–9 mo postpartum (7,16,17). A recently proposed strategy is to give two doses of 60 mg each to the mother instead of a single 60-mg dose as in the present study during the 8-wk period after delivery (18).

Maternal and immunization-linked infant supplementation, at the doses used, improved vitamin A status indicators at 6 mo in India and Ghana but the benefit at 9 mo was limited to some improvement in vitamin A stores only in India. Mortality reduction after vitamin A supplementation has been clearly demonstrated in infants and children older than 6 mo, but this benefit has not been shown consistently in younger infants. A study from Indonesia reported a reduction in mortality after supplementation to newborns (19), whereas two others in which infants were supplemented with or without maternal supplementation found no benefit (6,20). Increasing the immunization-linked vitamin A dose from 7.5 to 15 mg has also been proposed for improving vitamin A status between 6 and 9 mo of age. The efficacy of these higher doses in improving vitamin A status or mortality and morbidity is not yet known.

The maternal and infant supplementation strategy used here was more effective in reducing the risk of very low vitamin A stores, as indicated by higher MRDR cut-off values. The conventionally used MRDR cut-off value of >0.06 for identifying children 1–5 y of age with low vitamin A stores in developing countries is based on data from

TABLE

Overall and site-specific effect of maternal and infant vitamin A supplementation on vitamin A status of mothers given

	Overall			Ghana		
Indicator	Vitamin A	Placebo	Difference (95% CI)	Vitamin A	Placebo	Difference (95% CI)
Infant age 6 wk	*n* = 315	*n* = 309		*n* = 119	*n* = 123	
Serum retinol, *μmol/L*	0.65 ± 0.23	0.66 ± 0.22	−0.01 (−0.05, 0.03)	0.66 ± 0.23	0.69 ± 0.23	−0.03 (−0.08, 0.03)
Serum retinol ≤0.70 *μmol/L*	197 (62.5)	192 (62.1)	0.4 (−7.2, 8.0)	66 (55.5)	74 (60.2)	−4.7 (−17.1, 7.7)
Infants with MRDR	*n* = 291	*n* = 289		*n* = 99	*n* = 103	
>0.06	238 (81.8)	227 (78.5)	3.2 (−3.2, 9.7)	75 (75.8)	78 (75.7)	0 (−11.8, 11.8)
>0.09	151 (51.9)	156 (54.0)	−2.1 (−10.2, 6.0)	46 (46.5)	51 (49.5)	−3.0 (−16.8, 10.7)
>0.12	92 (31.6)	113 (39.1)	−7.5 (−15.2, 0.3)	26 (26.3)	36 (34.9)	−8.7 (−21.3, 4.0)
Infant age 6 mo	*n* = 332	*n* = 330		*n* = 118	*n* = 113	
Serum retinol, *μmol/L*	0.83 ± 0.29	0.80 ± 0.27	0.03 (−0.01, 0.08)	0.73 ± 0.27	0.68 ± 0.26	0.05 (−0.03, 0.11)
Serum retinol ≤0.70 *μmol/L*	101 (30.4)	122 (37.0)	−6.5 (−13.7, 0.6)	51 (43.2)	64 (56.6)	−13.4 (−26.2, −0.6)*
Infants with MRDR	*n* = 308	*n* = 308		*n* = 94	*n* = 93	
>0.06	136 (44.2)	163 (52.9)	−8.8 (−16.6, −0.9)*	41 (43.6)	50 (53.8)	−10.1 (−24.4, 4.1)
>0.09	51 (16.6)	84 (27.3)	−10.7 (−17.2, −4.2)*	18 (19.1)	26 (27.9)	−8.8 (−20.9, 3.3)
>0.12	20 (6.5)	46 (14.9)	−8.4 (−13.3, −3.6)*	4 (4.2)	10 (10.8)	−6.5 (−14, 1.0)
Infant age 9 mo	*n* = 293	*n* = 286		*n* = 92	*n* = 79	
Serum retinol, *μmol/L*	0.86 ± 0.31	0.85 ± 0.30	0.01 (−0.04, 0.06)	0.72 ± 0.28	0.74 ± 0.34	−0.02 (−0.11, 0.08)
Serum retinol ≤0.70 *μmol/L*	87 (29.7)	88 (30.8)	−1.1 (−8.6, 6.4)	44 (47.8)	39 (49.4)	−1.5 (−16.6, 13.5)
Infants with MRDR	*n* = 278	*n* = 266		*n* = 79	*n* = 61	
>0.06	103 (37.1)	97 (36.5)	0.6 (−7.5, 8.7)	36 (45.6)	24 (39.3)	6.2 (−10.2, 22.7)
>0.09	37 (13.3)	52 (19.5)	−6.2 (−12.5, −0.02)*	12 (15.2)	12 (19.7)	−4.5 (−17.2, 8.2)
>0.12	19 (6.8)	30 (11.3)	4.4 (−9.3, 0.4)	5 (6.3)	5 (8.2)	−1.9 (−10.6, 6.9)

[1] All values are means ± SD or *n* (%); * significant difference; $P < 0.05$. CI, confidence interval.

2

18–42 d postpartum on breast milk vitamin A status of lactating mothers in Ghana, India and Peru[1]

India			Peru		
Vitamin A	Placebo	Difference (95% CI)	Vitamin A	Placebo	Difference (95% CI)
n = 92	*n* = 86		*n* = 107	*n* = 99	
38.5 ± 23.4	28.2 ± 14.3	10.3 (4.6, 16.0)*	51.9 ± 24.5	45.4 ± 22	6.5 (0.02, 13.1)*
32 (34.8)	49 (57)	−22.2 (−36.5, −7.9)*	9 (8.4)	14 (14.1)	−5.7 (−14.4, 2.9)
n = 106	*n* = 107		*n* = 113	*n* = 113	
28.9 ± 15.2	24.9 ± 14.4	4 (−0.004, 8)	45.4 ± 19.8	48.8 ± 29.2	−3.4 (−10, 3.1)
63 (59.4)	71 (66.4)	−7 (−19.9, 6)	14 (12.4)	23 (20.4)	−8 (−17.6, 1.6)
n = 94	*n* = 104		*n* = 88	*n* = 112	
31.6 ± 18.4	32.3 ± 26	−0.7 (−7, 5.5)	49.9 ± 24.3	50.7 ± 27.1	−0.8 (−8, 6.3)
50 (53.2)	61 (58.6)	−5.5 (−19.3, 8.4)	14 (15.9)	17 (15.2)	0.7 (−9.4, 10.9)

Indonesia (10–12) and is higher than the >0.03 cut-off value used for U.S. children (21). There are no well-established norms for defining low vitamin A stores in infants in developing countries. Data from the current study and from a recent study in Bangladesh suggest that higher MRDR cut-off values of >0.09 or >0.12 are more specific indicators of low vitamin A stores in infants in developing countries (13).

The prevalence of subclinical vitamin A deficiency (serum retinol < 0.7 mmol/L) varied among the study sites and the effect of supplementation was related to the level of deficiency in the infants. It is therefore appropriate that multicenter studies of micronutrient supplementation be conducted either in countries with similar levels of deficiency or that these be designed to have adequate power to detect the effect at each site. The latter option would result in a greater generalizability of findings.

In conclusion, maternal and immunization-linked infant supplementation in the doses used modestly increased the vitamin A status of the infants at 6 mo of age. Additional strategies must be considered to avoid health consequences of vitamin A deficiency in the 6- to 9-mo period.

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60 mg retinol palmitate 18–42 d postpartum and infants given 7.5 mg at 6, 10 and 14 wk of age in Ghana, India and Peru[1]

India			Peru		
Vitamin A	Placebo	Difference (95% CI)	Vitamin A	Placebo	Difference (95% CI)
n = 98	*n* = 95		*n* = 98	*n* = 91	
0.57 ± 0.24	0.55 ± 0.23	0.03 (−0.04, 0.09)	0.70 ± 0.18	0.73 ± 0.17	−0.03 (−0.08, 0.02)
75 (76.5)	77 (81.1)	−4.5 (−16.0, 7.0)	56 (57.1)	41 (45.1)	12.1 (−2.1, 26.2)
n = 95	*n* = 95		*n* = 97	*n* = 91	
83 (87.4)	86 (90.5)	−3.2 (−12.1, 5.7)	80 (82.5)	63 (69.2)	12.4 (0.2, 24.6)*
70 (73.7)	75 (78.9)	−5.3 (−17.3, 6.8)	35 (36.1)	30 (33.0)	3.1 (−10.5, 16.7)
59 (62.1)	66 (69.5)	−7.4 (−20.8, 6.1)	7 (7.2)	11 (12.1)	−4.9 (−13.3, 3.6)
n = 97	*n* = 99		*n* = 117	*n* = 118	
0.91 ± 0.36	0.81 ± 0.29	0.1 (0.001, 0.19)*	0.88 ± 0.21	0.89 ± 0.20	0.01 (−0.06, 0.04)
25 (25.8)	35 (35.4)	−9.6 (−22.4, 3.2)	25 (21.4)	23 (19.5)	1.9 (−8.4, 12.2)
n = 97	*n* = 98		*n* = 117	*n* = 117	
61 (62.9)	75 (76.5)	−13.6 (−26.4, −0.9)*	34 (29.1)	38 (32.5)	−3.4 (−15.2, 8.4)
31 (31.9)	54 (55.1)	−23.1 (−36.7, −9.6)*	2 (1.7)	4 (3.4)	−1.7 (−5.8, 2.3)
16 (16.5)	35 (35.7)	−19.2 (−31.2, −7.2)*	0	1 (0.8)	—
n = 94	*n* = 94		*n* = 107	*n* = 113	
0.93 ± 0.36	0.87 ± 0.35	0.06 (−0.04, 0.16)	0.91 ± 0.24	0.91 ± 0.20	0.0 (−0.05, 0.06)
23 (24.5)	32 (34.0)	−9.6 (−22.5, 3.4)	20 (18.7)	17 (15.0)	3.6 (−6.2, 13.6)
n = 93	*n* = 94		*n* = 106	*n* = 111	
51 (54.8)	56 (59.6)	−4.7 (−18.9, 9.4)	16 (15.1)	17 (15.3)	−0.2 (−9.8, 9.3)
22 (23.6)	40 (42.6)	−18.9 (−32.1, −5.7)*	3 (2.8)	0	—
13 (14.0)	25 (26.6)	−12.6 (−24.0, −1.2)*	1 (0.9)	0	—

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