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Maternal vitamin A supplementation increases natural antibody concentrations of preadolescent offspring in rural Nepal

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ABSTRACT

Objective: B1a lymphocytes—which constitutively produce most natural antibodies (NAb)—arise from an early wave of progenitors unique to fetal life. Vitamin A regulates early lymphopoiesis. In animals, deficiency during this critical period compromises B1 cell populations. The aim of this study was to investigate the effect of maternal supplementation with vitamin A or β-carotene from preconception through lactation on NAb concentrations of offspring.

Methods: Participants (N = 290) were born to participants of a cluster-randomized, placebo-controlled trial of weekly maternal vitamin A or β-carotene supplementation (7000 μg retinol equivalents) conducted in Sarlahi, Nepal (1994–1997) and assessed at ages 9 to 13 y (2006–2008). Serum retinol was measured by reversed-phase high-performance liquid chromatography at mid-pregnancy and 3 mo of age. Enzyme-linked immunosorbent assay (ELISA) was used to measure children's plasma NAb concentrations at 9 to 13 y.

Results: Unadjusted geometric mean concentrations were 20.08 U/mL (95% confidence interval [CI], 17.82–22.64) in the vitamin A group compared with 17.64 U/mL (95% CI, 15.70–19.81) and 15.96 U/mL (95% CI, 13.43–18.96) in the β-carotene and placebo groups ($P = 0.07$), respectively. After adjustment, maternal vitamin A supplementation was associated with a 0.39 SD increase in NAb concentrations ($P = 0.02$). The effect was mediated by infant serum retinol in our statistical models. Although girls had 1.4-fold higher NAb concentrations ($P < 0.001$), sex did not modify the vitamin A effect.

Conclusions: In an undernourished population, maternal vitamin A supplementation enhanced NAb concentrations of preadolescent children. We posit that this was due to a greater allotment of B1a precursors during fetal life and a sustained higher count of NAb-secreting B1a cells.

Introduction

Vitamin A deficiency is widely prevalent in developing countries. A combination of inadequate diet, increased nutrient demand in pregnancy, and high parity place reproductive-aged women at particular risk. Globally, an estimated 19.1 million pregnant women have low serum retinol concentrations (<0.70 μmol/L) and, of these, roughly 10 million are affected by moderate to severe deficiency resulting in gestational night blindness [1]. Although the role of vitamin A in immune function is well accepted [2], little is known about the effect of early exposure to vitamin A deficiency on the emerging immune system. Data from Malawi indicate that infants born to mothers with low serum retinol had a threefold higher likelihood of mortality than those

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whose mothers had better vitamin A status [3]. In Nepal, gestational night blindness was associated with a 63% higher risk for death in the first 6 mo of life [4]. This risk was substantially reduced by weekly supplementation of mothers with low-dose vitamin A or β-carotene. Although the survival benefit of supplementation was limited to infants of night-blind mothers in this trial [5], it is possible that detecting alterations to the developing immune system in less severe cases of deficiency would require more sensitive functional indicators.

Lymphopoiesis may be particularly vulnerable to early insult [6]. Immune cells of the lymphoid lineage arise from distinctive waves of progenitor cells in the bone marrow. The most basic lymphocytes—the innate-like B1a cells and certain γδ T cells—are the products of an early wave of progenitors, unique to the fetal and neonatal period [7]. This endowment of long-lived early progenitor cells sustains innate-like lymphocyte populations at tightly regulated levels throughout life [8]. In B lymphopoiesis, the earliest developmental wave endows individuals with a long-lived, self-sustaining population of B1a lymphocyte precursors [7]. Unlike conventional B2 lymphocytes, B1a cells do not enter germinal centers or undergo affinity maturation. Rather, they constitutively produce most of the body's innate-like, low-affinity natural immunoglobulin (Ig)M with broad specificity for certain evolutionarily important epitopes [8]. Subsequent lymphopoietic waves give rise to the B1b and B2 cell populations, with an increasing capacity to recognize and refine antibody responses to specific antigens.

Vitamin A, and specifically its active derivative, retinoic acid (RA), is critical for early lymphopoiesis [9]. Deficiency in fetal mice has been shown to compromise the B1 cell populations [10]. We hypothesized that, in a vitamin A-deficient population, exposure to supplemental vitamin A in utero and via breast milk would permanently increase the allotment of long-lived B1a precursors. These precursors would sustain a larger B1a cell population and higher circulating NAb concentrations.

Materials and methods

Participants for this study were children, ages 9 to 13 y, born to women enrolled in the NNIPS (Nepal Nutrition Intervention Project-Sarlahi)-2 maternal supplementation trial conducted in the Sarlahi district of Nepal from 1994 to 1997.

The NNIPS-2 trial was a community-based, cluster-randomized, controlled trial designed to assess the effect of weekly supplementation with vitamin A or β-carotene from preconception through postpartum on maternal, fetal, and infant health and survival. Methods and results of the NNIPS-2 trial have been previously published [11]. Briefly, all married women living in the study area (270 wards) were considered eligible and verbal consent was obtained for their participation. Women were randomized by ward to receive one of three identical, coded supplements—vitamin A (7000 μg as retinyl palmitate), β-carotene (42 mg of all *trans*–β-carotene; 7000 μg retinol equivalents, assuming a 6-to-1 conversion ratio), or a placebo—designed to ensure they met their recommended dietary allowance (RDA) of vitamin A. Capsules were delivered on a weekly basis by a cadre of local women distributors, at which time information was also collected on pregnancy and vital status, menses in the past week, and treatment compliance. As distributors reported pregnancies back to the field office, trained fieldworkers were dispatched to the household to collect detailed information on mothers and their infants.

Data available from four home visits (early and late pregnancy; 3- and 6-mo postpartum) encompass household demographic characteristics, socioeconomic status (SES), anthropometry, maternal and infant dietary and morbidity histories, and other exposures (e.g., maternal substance use, tetanus vaccination, and strenuous work). Women in a subset of 27 contiguous wards consented to additional health and nutritional status assessments at the study clinic (mid-pregnancy and 3 mo postpartum) and at home within 2 wk after the child's birth (13 ± 11 d). Venous blood was collected from women at both clinic visits. A heel prick blood sample was taken from infants at the postpartum clinic visit. Processed samples were shipped in liquid nitrogen to the Center for Human Nutrition Laboratory in Baltimore and stored at −70°C until analyzed. Retinol and β-carotene concentrations were measured in serum and breast milk samples using reversed-phase high performance liquid chromatography, as previously described [12].

Children born to mothers enrolled in the NNIPS-2 trial were contacted again between 2006 and 2008 as part of a house-to-house follow-up study to evaluate longer-term developmental and functional outcomes. Fieldworkers collected information from mothers or primary caretakers on children's vital status, the presence or absence of 10 common morbidity symptoms over the previous week, usual and past week dietary intake, and SES. Fieldworkers also collected a 10-mL venous blood sample into sodium heparin-containing collection tubes. Samples were immediately returned to the project field laboratory and centrifuged at 1530*g* for 10 min at room temperature. Plasma was separated into four 1.5 mL aliquots, stored in liquid nitrogen, and shipped to the Johns Hopkins Bloomberg School of Public Health in Baltimore, Maryland on a semimonthly schedule for storage at −70°C. The present work was carried out in the plasma archive of children (N = 290) meeting our eligibility criteria:

1. First pregnancy tracked during the supplementation trial, although women may have had more than one pregnancy over the course of the trial,
2. Singleton birth, and
3. All biospecimens being available from both mothers during the original trial and children from the follow-up study.

The procedures of the original trial were reviewed and approved by the Joint Committee on Clinical Investigation at the Johns Hopkins School of Medicine, the Nepal Health Research Council in Kathmandu, and the Teratology Society in Bethesda, Maryland. The follow-up study protocol received ethical approval from the Institutional Review Board at Johns Hopkins and the Institute of Medicine at Tribhuvan University in Kathmandu, Nepal.

Laboratory analyses

Our hypothesis on B1a lymphopoiesis was developed after data collection had already been completed in the field. Thus, our analyses were limited to work with extant plasma samples. We proposed that, in this undernourished setting, maternal supplementation would yield a greater allotment of B1a precursors, sustaining a higher B1a cell count throughout life. As NAb are the unique and constitutively secreted protein products of B1a cells [8] and measureable in cryopreserved plasma, we used these as a proxy. There are numerous potential NAb specificities. We selected one specificity—IgM targeting double-stranded DNA (dsDNA)—that has been well-characterized as part of the germline-encoded, low-affinity NAb repertoire [13], which is detectable at regulated concentrations in normal human subjects throughout the life course [14,15]. We used a commercial sandwich ELISA to measure anti-dsDNA IgM in plasma samples from children at the time of follow-up (ALPCO Diagnostics, Salem, NH, USA; Catalog # 35-DSSHU-E01), using recombinant human dsDNA as a capture antigen and horseradish-peroxidase-conjugated antihuman IgM for detection. Human serum of known anti-dsDNA IgM concentrations (range 0–300 U/mL; functional sensitivity 1.0 U/mL) was used for calibration. We estimated intraassay precision using 10 replicates of one quality control sample. Interassay precision was calculated from quality control samples included on each plate. We ran all standards, controls, and samples in duplicate. Samples were rerun if the coefficient of variation (CV) of the pair exceeded 20%. The intraassay CV averaged 6.2% and the interplate CV was 14.3% (n = 9). As no international reference calibration is available for anti-dsDNA IgM, our findings were calibrated and data are presented using arbitrary units (U/mL).

We measured plasma concentrations of C-reactive protein (CRP) and α1-acid glycoprotein (AGP) as markers of current infection (CRP ≥5 mg/L or AGP ≥1 mg/mL [16]). For CRP, we purchased a high-sensitivity chemiluminescent immunoassay (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA; Catalog # LKCRP1) for the Immulite analyzer (Siemens Medical Solutions Diagnostics; Immulite 1000). This assay employs a solid-phase, anti–ligand-coated bead and a liquid phase consisting of a ligand-labeled monoclonal antihuman CRP antibody and alkaline phosphatase-conjugated polyclonal antihuman CRP. AGP was quantitated using a radial immunodiffusion assay procured from Kent Laboratories (Bellingham, WA, USA; Catalog #123511). Interassay precision was 12% and 3% for CRP and AGP, respectively. All laboratory analyses were carried out in the Center for Human Nutrition Laboratory at the Johns Hopkins Bloomberg School of Public Health in Baltimore, Maryland.

Statistical analyses

Our a priori hypothesis for this analysis was that children whose mothers received their weekly RDA of vitamin A or β-carotene would have higher plasma concentrations of anti-dsDNA IgM. We first tested for differences between supplement allocation groups in terms of maternal and household characteristics at baseline, treatment compliance, and characteristics of children at the time of

follow-up. We used analysis of variance and the χ^2 test for continuous and categorical variables, respectively. All analyses were carried out on an intent-to-treat basis. Between-group differences significant at $P < 0.2$ were flagged. We then used simple linear regression to assess relationships between those flagged variables and our continuous outcome. Variables that differed by supplement allocation and were also associated at $P < 0.2$ with NAb concentrations were considered in multivariate models.

We used multiple linear regression modeling to test the impact of maternal supplement allocation on NAb concentrations. Generalized estimating equations were used to derive standard errors to account for the NNIPS-2 cluster-randomized design [17]. Based on our univariate analyses, we included all variables that were associated with both maternal allocation group and NAb concentration ($P < 0.2$) as potential confounders in the multivariate models. Exceptions included maternal morbidity and both maternal and infant serum retinol concentrations, which were all strongly influenced by supplementation [11,12] and considered as potential mediating factors. A final, parsimonious model was generated using backward stepwise regression, retaining maternal supplement allocation and all variables significant at $P < 0.1$ in the full model.

Concerns have been raised that early life exposure to supplemental vitamin A may interact with childhood vaccination, and may have a differential impact on girls and boys [18]. A hypothesis has also been put forward that early life vitamin A supplementation may interact with immunization, acting as an adjuvant to amplify the nonspecific effects of vaccines [19]. We tested for effect modification by entering an interaction terms into the full model for child's sex. Although data were unavailable on exposure to childhood vaccinations, we did have information on whether mothers had received the tetanus toxoid vaccination during their pregnancy with the child subject. We used this information as a proxy to test for effect modification by vaccination in our models, assuming: 1) children whose mothers were vaccinated during pregnancy were likely to have been reached with childhood vaccinations and 2) maternal tetanus toxoid vaccination may itself be associated with nonspecific immune activation, to which the fetus was potentially exposed.

Model fit was tested by reviewing residuals for normality and extreme outliers, and by plotting residuals against predicted values to look for any deviations from a random scatter. Residuals were also plotted against all dependent variables, including those that had been removed from our parsimonious model. Finally, our previous analyses showed strong associations between supplementation and both maternal and infant serum retinol concentrations. As supplementation was expected to act by raising serum retinol concentrations, we employed serum retinol measures in sensitivity analyses. For these, we restricted our sample to mothers and infants in the placebo arm of the trial. We then generated models for each of the outcome variables using either maternal serum retinol concentrations during pregnancy or infant serum retinol at 3 mo postpartum as alternate primary exposure variables (i.e., in the place of maternal supplement allocation).

We used Stata Statistical Software: Release 10 (Stata Corporation, College Station, TX, USA 2007) for all statistical analyses. $P < 0.05$ was considered statistically significant.

Results

We identified 445 singleton, live-born children for whom complete clinical data were available from mid-pregnancy and early infancy (Fig. 1). Of these, 438 survived and were resident in the study area at the time of the 2006 NNIPS Cohort Follow-up census. We completed household interviews with 95% of the resident children (n = 418); however, 68 children were not met for biospecimen collection. An additional 20 children were excluded due to insufficient plasma. Our final sample size was 290 children, representing 66% of the surviving and resident children born to mothers enrolled in the NNIPS-2 trial. Follow-up rates did not differ by supplement allocation. As compared with the larger NNIPS-2 clinical subsample (with incomplete biospecimen archives) and the full NNIPS-2 sample, those in our study differed by caste (77% low caste or non-Hindu versus 87% and 86%, $P < 0.001$), radio ownership (37% compared with 32% and 28%, $P < 0.001$), and farmland ownership (35% owned >1 hectare compared with 21% and 20%, $P < 0.001$).

Maternal and household characteristics of our sample at the time of the original trial are summarized in Table 1. Although supplement allocation groups were comparable with respect to age, arm circumference, and other factors in the full trial sample [11], we found imbalances in baseline characteristics in the subsample eligible for this analysis. Differences suggested that households in the β-carotene arm may have been of somewhat higher SES. At the time of follow-up (Table 2), characteristics of the children born to trial participants were largely comparable across the three maternal supplement groups. All imbalances between groups were considered in our multivariate modeling.

Anti-dsDNA IgM concentrations were positively skewed and required natural log transformation to approximate a normal distribution. As such, we report all findings as geometric means. Geometric means of natural IgM concentrations at follow-up were 20.08 U/mL (95% confidence interval [CI], 17.82–22.64) in children born to vitamin A-supplemented mothers compared with 17.64 U/mL (95% CI, 15.70–9.81) and 15.96 U/mL (95% CI, 13.43–18.96) in those born into the β-carotene and placebo groups ($P = 0.07$; analysis of variance), respectively. After adjusting for minor differences between supplement groups at the time of follow-up, maternal vitamin A supplementation increased children's NAb concentrations by 0.34 SD (Fig. 2) compared with the placebo group ($P = 0.02$). Maternal β-carotene supplementation had no effect. Adding a variable for infant serum retinol to our model reduced the β-coefficient for vitamin A by roughly 20%, suggesting that the effect of maternal vitamin A supplementation was mediated, in part, by improving infant vitamin A status.

Although concentrations of anti-dsDNA IgM among girls were 1.4-fold higher than among boys ($P < 0.001$), there was no evidence that maternal supplement allocation had a differential effect by child's sex ($P = 0.99$). NAb concentrations also tended to be higher among children born to mothers who had received the tetanus toxoid vaccine during pregnancy ($P = 0.1$); however, our data did not support any effect modification by maternal vaccination status.

To test our findings, we first considered the potential influence of current infection. We found no correlation between natural IgM concentrations and either of the measured acute-phase proteins ($r = -0.03$, $P = 0.46$ for CRP; $r = -0.02$, $P = 0.73$ for AGP). Adding variables for CRP and/or AGP to our statistical models did not change the coefficients for β-carotene or vitamin A, and excluding children with elevated acute-phase proteins (CRP ≥5 mg/L; AGP ≥1 mg/dL; n = 80) strengthened the effect of vitamin A supplementation ($\beta = 0.31$, $P = 0.03$). We further assessed the relationship between children's NAb concentrations and the vitamin A status of their mother during pregnancy. This sensitivity analysis was restricted to the placebo group (n = 70), limiting our power. However, we did observe a nonsignificant direct relationship between natural IgM concentrations in preadolescent children and maternal serum retinol at mid-gestation (Fig. 3). Of note, supplementation with preformed vitamin A had a greater effect on maternal status than did β-carotene (0.18 ± 0.06 μmol/L versus 0.05 ± 0.05 μmol/L increase over placebo for vitamin A or β-carotene, respectively; simple linear regression), perhaps explaining the lack of increase in natural IgM among children born in the β-carotene group.

Discussion

Vitamin A is essential for fetal hematopoiesis, including the development of the B1a cell lineage during fetal and neonatal life [9,20]. B1a cells are the predominant source of NAbs [21–23], such as the anti-dsDNA IgM measured in this study. In a deficient population, we found that children whose mothers had received their weekly RDA of vitamin A throughout pregnancy and lactation had higher NAb concentrations in late childhood than

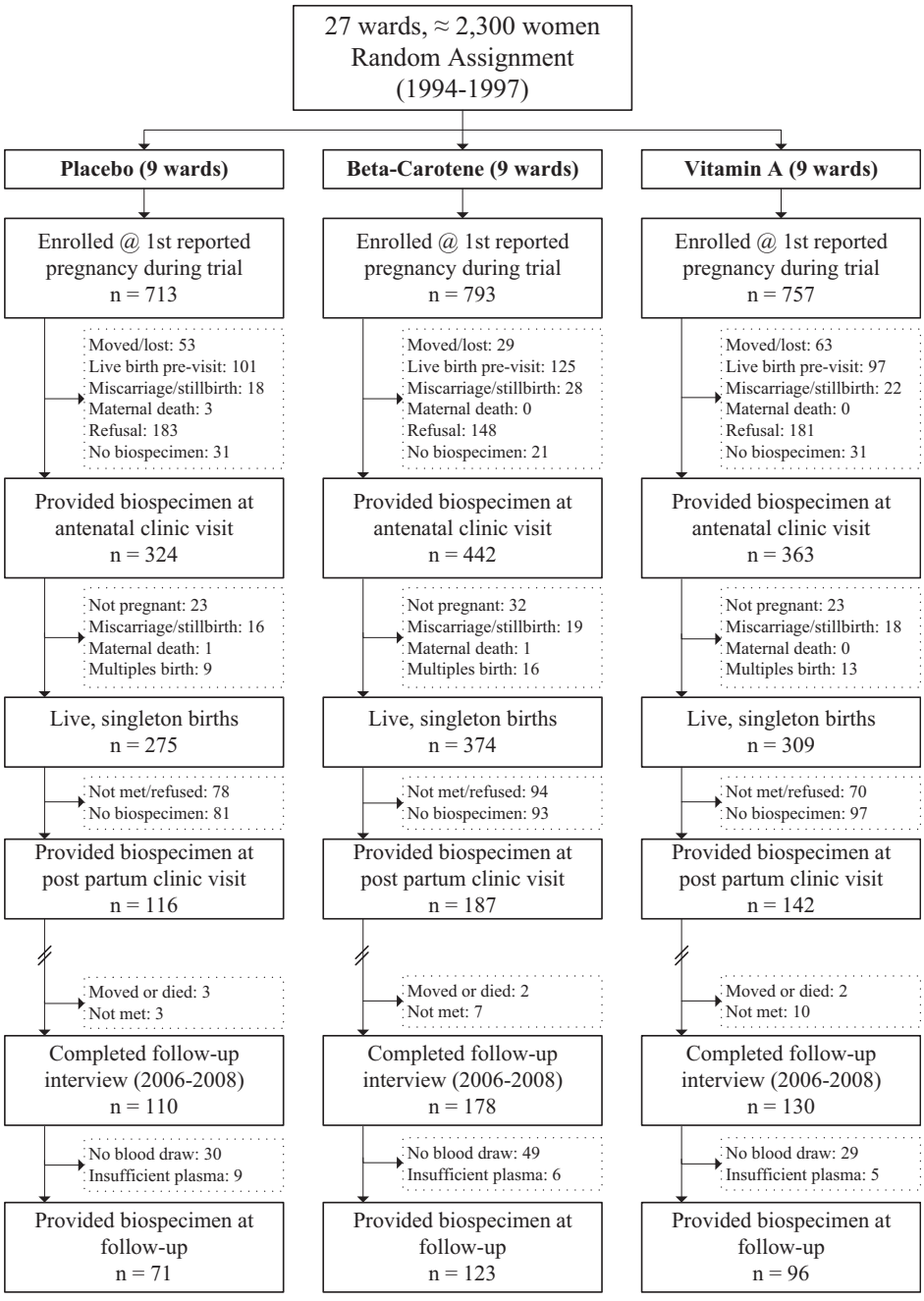

Fig. 1. Enrollment, participation, and losses to follow-up of children ages 9 to 13 y in rural Nepal by maternal supplement allocation. If participants had more than one pregnancy during the trial, only the first pregnancy was considered for the present analysis. Only children with a complete biospecimen archive were eligible for the present study. Biospecimens may have been unavailable due to refusals or insufficient sample volumes for analysis.

their peers. This effect was mediated, at least in part, by infant serum retinol concentrations. As such, it is possible that the null effect of maternal β-carotene supplementation was due to this supplement's lesser effect on infant serum retinol.

Higher NAb concentrations in these children may be the result of more efficient antibody production by B1a cells, which is known to be influenced by retinol status [24,25]. Alternatively, it may reflect a higher B1a cell count. The former explanation seems unlikely, as this would require a sustained higher intake of vitamin A among children born to supplemented mothers. Normalizing the vitamin A status of reproductive-aged women more likely protected the earliest wave of lymphopoiesis. Thus, our data are most consistent with a hypothesis that early life exposure to supplemental vitamin A ensured a larger or more robust population of early progenitor cells, sustaining a higher count of natural IgM-secreting B1a cells.

Our findings are supported by animal studies showing the critical importance of RA in hematopoiesis [26]. Although much of this work has focused on the myeloid lineage, some recent studies suggest a role for RA in regulating B lymphopoiesis. Treatment of cultured adult B lymphoid progenitors from healthy mice with all-*trans*-RA shortened the time required for their differentiation into CD19-positive cells [27], likely due to the role of RA in expression of transcription factors EBF1 and

Table 1
Characteristics of mothers and households at time of index pregnancy* in the NNIPS-2 maternal supplementation trial, by supplement allocation, Sarlahi, Nepal (1994–1997)

	Placebo n = 70 n (%)	β-carotene n = 121 n (%)	Vitamin A n = 96 n (%)
Maternal characteristics			
Age (y; n = 1 missing)			
<20	15 (21.4)	27 (22.0)	16 (16.7)
20–30	46 (65.7)	70 (56.9)	68 (70.8)
>30	9 (12.9)	26 (21.1)	12 (12.5)
Primiparous (n = 3 missing)	16 (22.5)	29 (23.8)	23 (24.5)
Arm circumference <21.5 cm (n = 7 missing)	42 (60.0)	58 (47.9)	55 (59.8)
Behavioral characteristics			
Strenuous work ≥2 d/wk	29 (40.8)	49 (39.8)	40 (41.7)
Smoked cigarettes (n = 5 missing; $P < 0.05$†)	5 (7.1)	25 (20.5)	20 (21.5)
Drank alcohol (n = 5 missing)	0 (0.0)	6 (4.9)	2 (2.2)
Compliance with supplementation ≥80% ($P < 0.01$)	53 (74.6)	70 (56.9)	71 (74.0)
Socioeconomic indicators			
Low caste or non-Hindu (n = 5 missing; $P < 0.001$)	63 (90.0)	78 (63.9)	78 (83.9)
Literate head of household (n = 5 missing; $P < 0.001$)	33 (47.1)	78 (63.9)	36 (38.7)
Asset ownership			
Radios (n = 5 missing; $P < 0.05$)	26 (37.1)	54 (44.3)	24 (25.8)
Goats (n = 5 missing)	47 (67.1)	70 (57.4)	51 (54.8)
Cattle (n = 5 missing)	55 (78.6)	87 (71.3)	62 (66.7)
Khet farmland, ≥1 hectares (n = 6 missing; $P < 0.001$)	35 (50.0)	47 (38.5)	18 (19.6)

* Participants may have had >1 pregnancy during the NNIPS-2 trial; only first reported pregnancies (index) were considered for the present study.
† All *P*-values based on $\chi 2$ test.

Pax-5 [10]. Although cultured fetal progenitors did not respond in a similar manner, the researchers did note a stark reduction in both B1a and B1b cells in mice exposed in utero and during the neonatal period to vitamin A deficiency [10]. This reduction could be corrected by RA [10]. We are not aware of any comparable research on the T lineage.

An impact of early vitamin A deficiency on lymphopoiesis may have relevance for the current debate regarding mechanisms of action for neonatal vitamin A supplementation [28–30]. Newborn dosing with vitamin A may act on the earliest lymphoid progenitors, boosting their differentiation into B1a or γδ T cells. These innate-like lymphocytes would provide crucial protection for neonates without prior exposure to common pathogens [8]. An impact primarily on the earliest wave of progenitors, versus the more transitional or adult-like progenitor cells arising during infancy, may further explain why the timing of dosing appears to modify the protective effect of neonatal supplementation [30]. Concerns regarding neonatal dosing have centered on their potential interactions with childhood vaccinations, particularly among girls [18,31]. We found no evidence that the effect of maternal vitamin A supplementation on NAb concentrations differed by child's sex, maternal tetanus toxoid vaccination (as a proxy for likely having received childhood vaccination), or both. However, girls and children whose mothers were vaccinated against tetanus toxoid did have higher NAb concentrations overall.

Table 2
Characteristics of children, ages 9 to 13 y, born to NNIPS-2 maternal supplementation trial participants, by supplement allocation, Sarlahi, Nepal (2006–2008)

	Placebo n = 70 n (%)	β-carotene n = 121 n (%)	Vitamin A n = 96 n (%)
Child characteristics			
Sex			
Male	38 (53.5)	67 (54.5)	46 (47.9)
Female	33 (46.5)	56 (45.5)	50 (52.1)
Age at follow-up (y)			
<11	14 (19.7)	36 (29.3)	28 (29.2)
11–12	42 (59.2)	65 (52.8)	50 (52.1)
>12	15 (107.1)	22 (61.1)	18 (64.3)
Height-for-age			
≥−2 Z-scores	31 (43.7)	52 (42.3)	46 (47.9)
<−2 Z-scores	40 (56.3)	71 (57.7)	50 (52.1)
Morbidity symptoms in week before blood draw			
Poor appetite, vomiting, or both	8 (11.3)	17 (13.8)	6 (6.3)
Diarrhea/dysentery	1 (1.4)	6 (4.9)	3 (3.1)
Fever	2 (2.8)	11 (8.9)	10 (10.4)
Productive cough, rapid breathing, or both	2 (2.8)	14 (11.4)	9 (9.4)
Painful urination	2 (2.8)	5 (4.1)	2 (2.1)
Ear discharge ($P < 0.05$)*	1 (1.4)	1 (0.8)	7 (7.3)

* Based on $\chi 2$ test.

A reduction in the B1a lymphocyte population and circulating natural antibodies has broad relevance. Natural IgM protect against encapsulated bacteria such as *Streptococcus pneumonia* [32], a major cause of morbidity and mortality in the developing world. These innate-like defensive proteins are produced irrespective of age or prior pathogen exposure and are therefore particularly important in early infancy [23]. Emerging research is also highlighting the role of natural antibodies in responding to malignant cells [33] and in "housekeeping" after oxidative stress-induced damage or conventional apoptosis [34]. Reductions in B1a cells and natural IgM can therefore have implications for immune-mediated or inflammatory diseases in later life. This is evidenced by epidemiologic studies linking low concentrations of certain NAb specificities with an increased risk for cardiovascular disease [35] and stroke [36].

The major strength of this study was the nature of the exposure, in which women were randomized by cluster to one of three trial arms. The NNIPS-2 trial was also unique in that supplements were delivered from before conception through lactation, presumably assuring exposure to vitamin A or β-carotene throughout the full critical period of early lymphopoiesis. We had access to an extensive database of information regarding maternal/fetal health and nutrition from early pregnancy through the time of birth, as well as information about mothers and their infants throughout the postpartum period. Additionally, we were able to recontact, interview, and collect biospecimens from almost 70% of children born during this trial more than 10 y later. However, our study does have limitations. Given our requirements for biospecimens

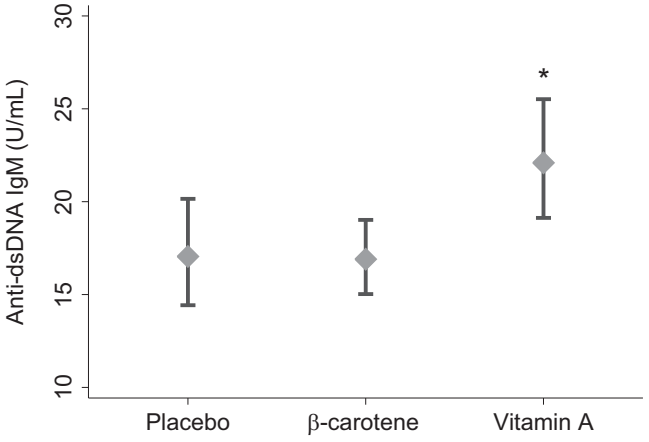

Fig. 2. Effect of maternal vitamin A or β-carotene supplements delivered weekly from preconception through postpartum on natural antibody concentrations of children ages 9 to 13 y in rural Nepal (n = 250). Values are geometric means (±95% confidence intervals), adjusted for the design effect, maternal vaccination during pregnancy, current child ear discharge status, and household assets (mobile phone, farmland). *Significantly different from placebo, $P < 0.05$. IG, immunoglobulin.

from mothers and their infants or children during pregnancy, postpartum, and later in life, our sample was not fully representative of the original trial. This highly compliant subsample tended to be of better SES. We also found minor differences between supplementation allocation groups in our subsample that were not apparent in the larger trial [11]. Although we attempted to control for these factors in our models, there is the possibility of some residual confounding.

The primary limitation of our study was in characterizing early lymphopoiesis. As this hypothesis was not posed until after our field data collection was completed, we were limited to measuring markers in cryopreserved plasma samples. NAb concentrations have not been previously validated as a marker of the B1a population. However, NAb are constitutively produced by B1a cells and are thought to be protein products unique to these cells [37]. We measured only one NAb specificity: anti-dsDNA IgM. Unlike anti-dsDNA IgG, the IgM isotype is protective against lupus-associated pathology [38], in support of it being a natural rather than pathogenic antibody. It is possible that anti-dsDNA IgM may not be representative of total NAb concentrations. Future studies should directly measure counts of innate-like B cells. This work should also be extended to the T lineage, where human studies reveal a strong regulatory bias of fetal stem cell–derived T lymphocytes [39].

Given the self-sustaining nature of fetal progenitors and their sensitivity to vitamin A nutriture, early life exposure to vitamin A deficiency could have a lasting impact on their progeny—the innate-like lymphocyte populations—and the protection that they afford. Although our work focuses on vitamin A, this can be viewed as a sentinel nutrient. Gestational deficiencies or excesses in other essential nutrients may also influence the rapidly developing immune system, meriting further research on nutritional modulation of early lymphopoiesis.

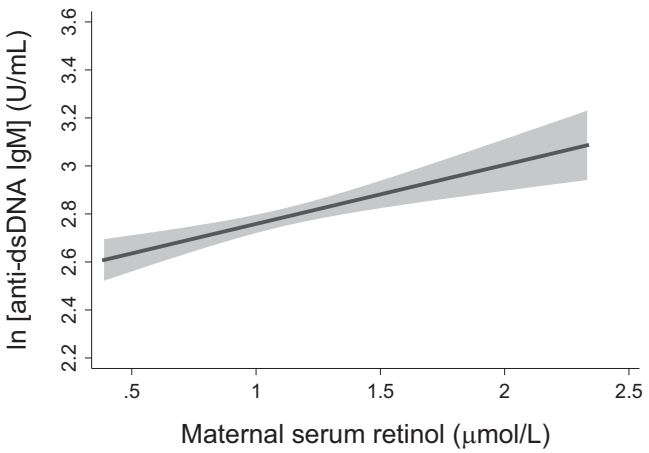

Fig. 3. Relationship between natural antibody concentrations of children ages 9 to 13 y in rural Nepal and maternal serum retinol concentrations at mid-pregnancy (placebo group only; n = 70), adjusted for maternal fruit and vegetable intake during pregnancy. Fit (line) and confidence intervals (shaded area) of multiple linear regressions with generalized estimating equations. Ig, immunoglobulin.

Conclusion

In an area of endemic vitamin A deficiency, we showed that exposure to supplemental vitamin A in utero and during lactation increased children's NAb concentrations. We posit that this results from a greater allotment of B1a precursors during fetal life and a sustained higher count of NAb-secreting B1a cells.

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