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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