Published in final edited form as:

J Pediatr Gastroenterol Nutr. 2010 March ; 50(3): 309–315. doi:10.1097/MPG.0b013e3181a96489.

Vitamin A supplementation effects on intestinal barrier function, growth, total parasitic and specific *Giardia* spp. infections in Brazilian children: a prospective randomized, double-blind, placebo-controlled trial

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Abstract

Background—This study evaluates the effects of retinol on intestinal barrier function, growth, total parasites and *Giardia* spp. infections in children in the Northeast of Brazil.

Methods—The study was a double-blind, randomized placebo-controlled trial (<http://clinicaltrials.gov;Register#NCT00133406>) involving 79 children received vitamin A 100,000 - 200,000 IU (n = 39) or placebo (n = 40) at enrollment, 4 and 8 months, followed for 36 months. Intestinal barrier function was evaluated using the lactulose:mannitol test. Stool lactoferrin was used as a marker for intestinal inflammation.

Results—The groups were similar with regard to age, sex, nutritional parameters (z-scores), serum retinol concentrations, proportion of lactoferrin positive stool samples, and intestinal barrier function. The lactulose:mannitol ratio did not change during the same time of follow-up (p>0.05). The proportion of lactoferrin positive samples evaluated at one month did not change between groups (p>0.05). Total intestinal parasitic specifically new infections were significantly lower in the vitamin A treatment compared to control group; these were accounted for entirely by significantly fewer new *Giardia* infections in the vitamin A treatment group. The cumulative z-scores for weight-for-length or height (WHZ), length or height-for-age z-scores (HAZ), and weight-for-age (WAZ) did not change significantly with vitamin A intervention for 36 months of follow-up.

Conclusions—These data showed that total parasitic infection and *Giardia* spp. infections were significantly lower in the vitamin A treatment group when compared to the placebo group, suggesting that vitamin A improves host defenses against *Giardia* infections.

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There is no Conflict of interest.

Part of this manuscript was presented at the 55th Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 12-16, 2006, Atlanta, GA, Abstract # 782.

Keywords

Retinol; intestinal barrier function; intestinal parasites; inflammation; diarrheal diseases; growth

INTRODUCTION

The role of vitamin A in the immune response, maintenance of mucosal cells and overall development is well accepted. Although vitamin A supplementation in children under five years old from deficient populations reduces all causes of mortality by 30% (1,2), its effects on diarrheal disease morbidity remain controversial. In a meta-analysis reported by Grotto et al. (3), high doses of vitamin A supplements had no consistent overall protective effect on the incidence of diarrhea and were not recommended as a routine intervention for all children. However, a recent study showed that the beneficial effect of vitamin A supplementation in reducing watery diarrhea and respiratory infections was dependent on socio-economic variables and nutritional status (4). These variable effects of vitamin A supplementation necessitate further studies to assess diarrheal diseases, malnutrition and the unique biological processes associated with vitamin A supplementation.

The immunological bases that explain the impact of vitamin A supplementation are poorly understood. Animal models have shown that vitamin A deficiency is associated with a switch from Th2 immune responses, which are important for the resolution of helminthic infections to a Th1 response, crucial for the resolution of intracellular infections. The expression of the interferon gamma (IFN- γ) gene is down regulated by retinoic acid, the active form of retinol in cells. Studies in murine model have shown that vitamin A supplementation increased IL-4, IL-5 and IL-10 and decreased IFN- γ production (5,6). Similar effects of vitamin A in humans are less clear.

The effects of vitamin A supplementation on gut integrity are also not well established. Previous cross-sectional investigations in populations of children with high rates of subclinical vitamin A deficiency (VAD) have shown that serum retinol concentrations are inversely correlated with intestinal permeability, as measured by the lactulose:mannitol absorption ratio, suggesting an important role of vitamin A in intestinal barrier function (7–9). However, the time-course of the effects of vitamin A supplementation on intestinal barrier function, growth and short- and long-term adverse effects in children require further investigation. No previous studies have investigated the role of vitamin A supplementation in reducing intestinal inflammation and only a few have examined the impact of vitamin A on specific intestinal pathogens and disease morbidity (10,11).

The impact of vitamin A intervention on growth and development also remains controversial (9,12,13). In a randomized clinical trial, Hadi et al. (2000) reported that a group of children less than five years old, with serum retinol $<0.35 \mu\text{mol/L}$, who received vitamin A had significant increases in height and weight compared with a placebo control group (12). However, no growth response to vitamin A was observed in children with an initial serum retinol concentration $\geq 0.35 \mu\text{mol/L}$. Villamor et al, (2002) also showed that vitamin A supplementation ameliorates the adverse effects of HIV-1, malaria, and diarrheal diseases on child growth (13). Therapy with vitamin A and zinc was associated with significantly improved linear growth in Brazilian children with evidence of subclinical VAD (9).

Findings from previous investigations warrant further studies to evaluate the effects of vitamin A supplementation on intestinal barrier function, inflammation, disease morbidity, intestinal parasitic infections and growth in children in developing areas. We hypothesize that vitamin A supplementation can prevent or ameliorate damage to the intestinal barrier, reduce

inflammation, decrease intestinal parasitic infections and diarrhea disease and other morbidity and improve growth in children at high risk for subclinical VAD in an impoverished urban community in Fortaleza, Ceará, in the northeast of Brazil.

MATERIALS AND METHODS

Ethics

The study protocol and informed consent were approved by Institutional Review Boards (IRB) including the Federal University of Ceará (UFC), Fortaleza, Ceará, Brazil, the University of Virginia and the Data Safety and Management Board (DSMB) at the National Institute of Health.

Geographic location and study population

The study was conducted in Fortaleza (3°46' 48.00" South and 38°35' 24.00" West), the capital of the Ceará state in the Northeast of Brazil. The estimated population in Fortaleza for the year of 2007 was 3,097,641 and the infant mortality is 35/1000 live births. The study population belongs to an impoverished urban community called Parque Universitário (3°44' 58.27" South and 38°34' 30.80" West) that is located approximately 5 km from the headquarters and laboratories at Clinical Research Unit & Institute of Biomedicine/Center for Global Health (UPC & IBIMED/CGH; www.upcibimed.ufc.br), School of Medicine, UFC. A census done in 1998 at Parque Universitário showed a total population of 3,541 inhabitants, of whom 957 (27%) were children less than nine years old. Parents or guardians of children were invited to participate after informed consent. The intervention study commenced enrollment in June 2000 and finished in August 2004.

Study design and protocol, eligibility and exclusion criteria for enrollment of subjects

The study was a prospective, double-blind, randomized, placebo-controlled trial (phase III). The parent or guardian of the children, field study team and investigators were blinded with regard to the treatment agent. After the community census, a total of 324 children were assessed using the surveillance system as described below, for eligibility and 79 children were randomized (using computer-generated random numbers) to receive placebo (tocopherol 40 IU; N = 40) or vitamin A group (retinyl palmitate; 100,000 IU for children <12 months and 200,000 IU for children at least 12 months old; N = 39) and treated every four months. Tocopherol was chosen as placebo for vitamin A group because it is also a fat-soluble vitamin and tocopherol dosing preparation is similar to vitamin A capsule dosing. This way the study could be kept double-blind. Thus children received the first supplementation at enrollment, a second dose after 4 months, and a final dose at 8 months (Figure 1). All treatments were directly observed by the trained healthcare agents (HCAs). The HCAs were blinded to the intervention groups. Vitamin A and placebo capsules were the same color, size and taste, and were kindly provided by Hoffmann-La Roche, S.A. (Basilea/Suiza). The thirty six months follow-up protocol is summarized in Figure 1. The age range for these children was chosen because the prevalence of vitamin A deficiency in the Northeast of Brazil population is greatest in children less than nine years old (14).

The eligibility criteria for enrollment were as follows: (a) children from 2 months to nine years old with length or height-for-age score (HAZ) less than the median (−0.06) for the Parque Universitário community; (b) be a resident in this urban community; and (c) parental or guardian consent. The exclusion criteria were: (a) being exclusively breast-fed; (b) participants in other study in the past two years; and (c) being ill with fever > 38 °C at time of enrollment. This was chosen to increase the enrollment of children with marginally low levels of retinol and more prone to malnutrition in this region.

Household surveillance, disease morbidity, anthropometric measurements, sample collection and handling

Surveillance was done twice weekly by household visits with the parent or guardian responsible for each child by a field team, including one nurse coordinator and three healthcare agents. The occurrence and frequency of diarrhea and other illnesses (adverse events) e. g., pneumonia, asthma, bronchitis, etc were recorded. Diarrhea was defined as three or more liquid stools in the past 24 hours. An episode of diarrhea was defined as two or more days of diarrhea with no more than a 48 hour interval without diarrhea.

A calibrated digital weighing scale with 100g precision (Tanita Solar Scale, Tanita Corporation of American Inc., Arlington, IL) was used to measure the weight of each child. All children were asked to wear light clothing for this measurement. Length or height was measured in the supine position for children under 24 months old and standing for children aged 24 months or older. All measurements were done with an anthropometric rod with increments of 0.1 cm.

Stool samples for lactoferrin and pathogens were collected at enrollment and at 1 month of the study protocol (Figure 1). A sample of blood, approximately 5 ml, was collected at study entry in a serum separator tube without anticoagulant from the first 23 children and carried to the laboratory with proper precautions to prevent light exposure for vitamin A determination. This sub-set of blood samples was used to evaluate vitamin A deficiency as described in the results. All samples were properly carried within 3 hours to the nearby laboratories at UPC & IBIMED/CGH, UFC, and frozen at -80°C until the time of analysis. A portion of the fresh stool samples were used for initial microbiology procedures, testing for lactoferrin, and direct microscopy and stool concentration examining for ova and parasites as previously reported (15). A monoclonal ELISA for *Giardia* spp. and *Cryptosporidium* spp. from TechLab (Blacksburg, VA) was used according to the manufacturer's instructions. Fecal lactoferrin was measured using an ELISA method (IBD-Scan, Techlab, Inc., Blacksburg, VA) and following instructions provided in the kit assay.

In order to explore specific immunological markers in both groups, placebo and vitamin A, we measured four cytokines by ELISA in the available stool samples stored with two protease inhibitors (Soy trypsin inhibitor and phenylmethylsulfonyl fluoride; both in a concentrations of 1 mg/mL) at the time of collection. These cytokines were used to define markers for intestinal Th1 response (IFN-gamma and TNF-alpha; Biosource Immunoassay kits, Camarillo, CA) and Th2 response (IL-4, Biosource Immunoassay kits, Camarillo, CA; and IL-10, Biosource Europe S.A., Nivelles, Belgium).

Adverse event and serious adverse event surveillance

The adverse event and serious adverse event surveillance system was developed by NIH/NIAID/DMID and used according to the guidelines of the US NIAID Division of Microbiology and Infectious Diseases and procedures detailed as good clinical practice (16,17). An adverse event was defined as any untoward medical occurrence that arose during administration of vitamin or placebo and that may or may not have a causal relationship with the study agent. A serious adverse event was defined as any adverse experience that resulted in any of the following outcomes: death, threat to life, requirement for inpatient hospitalization, persistent or significant disability or incapacity, or an important medical event.

Serum retinol determinations

Serum retinol concentrations were measured by a high pressure liquid chromatography (HPLC) method as described elsewhere (18,19). Briefly, retinyl acetate was used as an internal standard for assessing the recovery of retinol and the assay variability range was 3–6%.

Intestinal permeability test

A sample of urine for lactulose and mannitol determination was collected at the end of the intestinal permeability test as described below, at enrollment, 1 month, 1.5 months and 4 months (Figure 1). Methods used to determine urinary lactulose and mannitol levels were the same as previously reported (20).

Statistical analyses

Sample size calculations, using a power of 90% and a two-sided significance level of 5%, were done using previous data available from the same population in the lactulose:mannitol ratio and anthropometric z-score parameters. The estimated sample size of at least 35 for each treatment group was calculated for the intervention study assuming a 30% loss to follow-up. Z-scores for length or height, weight and weight-for-length or height were calculated using EpiInfo Nutstat program software version 6.0 (Centers for Disease Control and Prevention, Atlanta, GA).

Parametric (Student's *t* tests) and non-parametric tests (Mann-Whitney tests, Chi-square tests or Fisher exact tests) were used when recommended to compare differences between treatment groups. Covariance analysis (ANCOVA) was used to adjust the influence of age and seasonality when comparing parameters between vitamin A and placebo groups. In addition, Pearson linear correlation coefficients were used to test the association of intestinal barrier function parameters and anthropometrics measurements after adjusting for age. All statistical analyses were performed using the Statistical Package for Social Sciences version 11.5 (SPSS Inc. Chicago, IL). A statistically significant difference was accepted when the alpha value was 0.05 or less.

RESULTS

The flow diagram of the study population and activities during the twelve month period are summarized in Figure 1. A total of 324 children were screened (< -0.06 median length or height-for-age) and had parental or guardian informed consent. After ten withdrew or moved, three hundred and fourteen were available to enroll in the study (this also included two zinc and glutamine arms that are being reported separately). A total of 79 children were randomized, of which, 39 children were randomized to the retinol group and 40 to the placebo group. After twelve months follow-up, a total of 22 children were withdrawn from the study for the following reasons: (a) change of address (16); (b) parents or guardians did not cooperate with the study (5); and (c) one had above the median z-score for length or height at the time of the study initiation. The percent completing the study at twelve months was 72.2%, a little higher than the expected 30% drop-out rate estimated at the beginning of the study. The drop-out rate was similar in both groups ($p > 0.05$).

The characteristics of children by age, sex, nutritional status, initial serum retinol concentration, fecal lactoferrin and lactulose:mannitol ratio are summarized in Table 1. This population had a mean age and standard deviation of 43.3 ± 27.7 months and there was no significant difference between the retinol group versus placebo control. A total of 42 (57%) were male and both groups were similar in gender proportions ($p > 0.05$). Nutritional status, as measured by z-scores for length or height (stunting), weight and weight-for-length or height (wasting) was not different between these two groups of children.

The prevalence of vitamin A deficiency in this population of children was well characterized in a recent paper by Viera et al. (2008) (23). In this study we chose approximately 30% (23/74) of the total samples to measure retinol concentrations. The result on the prevalence of vitamin A deficiency was similar to this previously cited paper. Thus, seventy four percent (17/23) of

the children had normal serum vitamin A concentrations ($>1.05 \mu\text{mol/L}$) at study commencement. The prevalence of children with insufficient serum vitamin A concentrations (retinol $\leq 1.05 \mu\text{mol/L}$) was 26% (6/23) with 22% (5/23) with mild deficiency ($0.36 - 1.05 \mu\text{mol/L}$), only one (4%) was moderately deficient ($0.36-0.71 \mu\text{mol/L}$) and none were severely deficient ($<0.36 \mu\text{mol/L}$) (Table 1).

The frequency of lactoferrin in stool samples from these children was 23% (14/60) overall. The proportion of children with positive lactoferrin in stool samples was similar in the vitamin A compared to placebo group at the first day of the study protocol (Table 1) and at one month follow-up (33%; 7/21 versus 31%; 9/29; $p > 0.05$). Lactulose:mannitol ratio did not differ between groups (median/minimum-maximum: 0.0690/0.0080-0.7100 versus 0.0890/0.0050-1.1250; $p > 0.05$) (Table 1). The total median and range for this population was considered normal compared to a healthy population from the same geographic area (18).

Figure 2 shows repeated measurements at standard intervals in the same subjects of the intestinal barrier function parameter, lactulose:mannitol ratio, by treatment group during the first four months of follow-up. The lactulose:mannitol ratio did not change significantly between vitamin A and placebo groups during the four month follow-up period ($p > 0.05$) (Figure 2). There was a consistently lower percentage of urinary lactulose excretion among the vitamin A group than in the placebo group, and this difference was statistically significant at four months of follow-up (median/minimum-maximum: 0.21/0.0400-1.2800 versus 0.74/0.0200-3.0500; $p = 0.042$). The percentage of urinary mannitol excretion followed the same pattern, with differences between vitamin A and placebo groups reaching statistical significance at four months of follow-up (3.06/0.06-34.46 versus 8.25/0.19-32.13; $p = 0.008$).

Samples for parasitic infections were collected at enrollment and one month after initiation of the protocol. A total of 63 samples (85%; 63/74; 33 in the placebo group and 30 in the vitamin A group) at enrollment and 56 samples (79%; 56/71; 31 in the placebo and 25 in the vitamin A group) at one month into the study. Thus, stool samples were 85% and 79% collected and available for examination at enrollment and at one month of the study protocol, respectively. At least one parasite was found in 19 (29%) of all samples and two or more were found in 6 (10%) samples at study enrollment of children in the study protocol. The most frequent parasitic infections at enrollment were as following: (a) *Ascaris lumbricoides*, 10 (16%); *Trichuris trichiura*, 8 (13%); (c) *Giardia lamblia*, 2 (3%); (d) *Entamoeba coli*, 2 (3%); and (e) *Hymenolepis diminuta*, 1 (2%). There was no significant difference in the total and specific proportions of parasitic infections at enrollment between vitamin A versus placebo group ($p > 0.05$). The frequency of parasitic infections at one month follow-up was as following: (a) *Ascaris lumbricoides*, 10 (18%); *Trichuris trichiura*, 7 (13%); (c) *Giardia lamblia*, 6 (11%); (d) *Entamoeba coli*, 1 (2%); (e) *Hymenolepis diminuta*, 1 (2%); and *Strongyloides stercoralis* 2 (4%). The vitamin A group (5/25; 20% versus 14/31; 31%) had significantly fewer total parasitic infections ($p = 0.048$) and specifically *Giardia lamblia* infection (0/25; 0% versus 6/31; 19.4%; $p = 0.028$) at one month of the study protocol (Figure 3). Additional tests were done on available stool samples using antigen detection by a monoclonal ELISA for *Giardia* spp. and *Cryptosporidium* spp. from TechLab (Blacksburg, VA). The results using this test for *Giardia* spp. showed a sensitivity of 83% (5/6), specificity of 96% (47/96), positive predictive value of 71% (5/7), negative predictive value of 98% (47/48) and prevalence of 11% (6/56). A positive exam for *Giardia* spp. was considered when both tests were positive and the results showed again a consistent reduced frequency of *Giardia* spp. infection associated with vitamin A (0%; 0/11) versus placebo group (33%; 5/15; $p = 0.048$). There were no *Cryptosporidium* spp. positive samples as also evidenced before by direct stool observation with modified acid fast stain.

The results on cytokines showed no significant difference between placebo (IFN-gamma: 29.4 ± 6.38 pg/mL; TNF-alpha: 6.1 ± 1.18 pg/mL; IL-4: 5.3 ± 0.75 pg/mL; and IL-10: 2.9 ± 1.72 pg/mL; N = 22) and vitamin A (IFN-gamma: 32.3 ± 8.98 pg/mL; TNF-alpha: 9.0 ± 1.88 pg/mL; IL-4: 7.6 ± 3.10 pg/mL; and IL-10: 5.7 ± 2.40 pg/mL; N = 20; $p > 0.05$) regard to intestinal markers for Th1 or Th2 response.

Cumulative anthropometric z-scores, adjusted for age and season during the thirty six months of follow-up for the vitamin A and placebo groups are summarized in Table 2. The time course of cumulative anthropometric z-scores for weight-for-height, height-for-age, and weight-for-age did not significantly change over 24 or 36 months of follow-up when vitamin A and placebo were compared ($p > 0.05$). There were transient decreases in the cumulative HAZs at 8 and 12 months follow-up in the vitamin A treatment group compared to the placebo control group (Table 2). The linear growth modeling for repeated measures was also done and it showed only transient decrease in the HAZs at 12 month ($p = 0.0312$) follow-up in the vitamin A group compared to control.

Adverse events and serious adverse events were followed during only twelve months. A total of 31 (39%) adverse events and 14 (18%) serious adverse events were documented during the study period. The most common adverse events were respiratory infections (n=11), diarrhea (n=5), asthma (n=5), pneumonia (n=4) and others (bronchitis, furuncles, impetigo, fever, tonsillitis and hordeolum), one for each adverse event. The proportion of adverse events was similar in both groups ($p > 0.05$). Serious adverse events included asthma (5), pneumonia (4), diarrhea (3) and respiratory infections (2) and the proportion was also similar in both groups ($p > 0.05$) and none were associated with vitamin A treatment or placebo by the investigators team as well as the Data and Safety Monitoring Board (DSMB) reviewed as noted on method section.

DISCUSSION

In this double blind clinical trial we examined the effect of vitamin A supplements on intestinal permeability, intestinal infestation and growth in children with marginal vitamin A status in a developing country. An earlier study by Chen et al. (2003) showed that increased lactulose:mannitol ratio was associated with reduced serum retinol concentration (9). This effect was also seen by Quadro et al. (2000) in severely malnourished hospitalized children (7). A study of healthy infants of weaning age were administered eight weekly doses of 5 mg orally retinol and hospitalized infants received one large oral dose of 60 mg (200,000 IU) (24). They observed improvement in gut integrity in both studies. Filteau et al. reported that infants from HIV-infected women in South Africa did not improve gut integrity after maternal vitamin A supplementation (25). However, they observed a trend toward improvement of gut integrity in infants that later got HIV infection and this was most likely due to an effect of vitamin A on tight junctions as indirectly measured by the lactulose marker (25). Rollins et al. showed that children with severe diarrhea did not improve their gut integrity after three days of taking retinol by random assignment (26). The lactulose:mannitol ratio data showed in this report also suggest that there is no significant beneficial effect of vitamin A on gut integrity, despite the trend toward lower lactulose as noted by Chen et al. (9).

Although we had the fecal parasites evaluated only at one month, the results reported showed a significant decrease in the prevalence of intestinal parasitic infection, especially *Giardia* spp., in children treated with vitamin A compared to placebo control. Three recent reports showed that retinoic acid from the catalysis of vitamin A modulates gut-associated dendritic T cells into regulatory T cells and direct these cells to home to the gut lamina propria and epithelial cells (27–29). Furthermore, this modulation of retinoic acid was associated with transforming growth factor (TGF- β) in the gut-associated dendritic cells. The cytokines results reported in

this study did not show evidence of Th1 or Th2 response on vitamin A versus placebo group. A recent study in mice (30) showed that *Giardia muris* infection induced the same secreting IL-4 and IFN-gamma and there was an increased number of CD4 cells in Peyer's patches. Collectively their data showed that a significant cellular and humoral immune response is important against *Giardia* cyst antigens. Taken altogether, these data from murine studies and our studies in this report, we hypothesize that vitamin A may stimulate CD4 cellular immune and/or humoral immune antibody responses to reduce *Giardia* infections. Further studies will be needed to explore mucosal cytokines secreting T-lymphocytes and local antibodies such as specific IgA and IgG in the intestinal fluid in order to explain in detail this potential vitamin A effect on T-cell intestinal response and *Giardia* spp. infection.

A recent intervention study with vitamin A in children showed a reduction on the prevalence of enteropathogenic and enterotoxigenic *Escherichia coli* infections, as well as shorter duration of these infections (10). This intervention did not show a significant reduction in the prevalence of *Giardia* spp., but they used a smaller vitamin A dose and probably there is also difference in the prevalence of VAD between these two population studies. The decrease in the prevalence of intestinal parasitic infection may indirectly modulate the beneficial effect of vitamin A treatment on the paracellular pathway, showed by the decrease on the lactulose marker permeation, seen in the results of this current study.

Despite several studies of vitamin A supplementation on child growth, a causal relationship between vitamin A and growth in children still has not been conclusively established (9,12, 13). Previously, studies have shown that vitamin A supplementation improves growth (9,31), but others have shown no effect (32,33). Two reports have shown that growth improvements with Vitamin A supplementation were dependent on the severity of vitamin A deficiency and presence of other conditions such as HIV infection in children in developing countries (12, 13). The present double-blind, randomized, placebo-controlled trial evaluated the effect of vitamin A supplementation on cumulative growth after adjusting for age and season in children with growth deficits, but normal to moderately deficient serum retinol levels. Vitamin A supplementation did not change the cumulative weight-for-length or height, length or height-for-age or weight-for-age z-scores for a thirty-six-month period in this population; however, we observed a transient decrease in HAZ at 12 month for vitamin A group compared to controls. This study had a limitation to evaluate the long-term benefit from multiple doses of vitamin A supplementation in children with normal or only marginal VAD due to a small sample size. This intervention study also evaluated potential adverse events, serious adverse events and the occurrence of all illnesses over the twelve month treatment period. There were no adverse or serious adverse events associated with multiple doses of vitamin A evaluated for twelve months of observations. The diarrhea and respiratory illness morbidity were not different between these two groups, but the sample size was not designed to examine for these morbidities as well as adverse events.

In conclusion, multiple doses of vitamin A did not change long-term evaluation of weight-for-length or height, length or height and weight z-scores. The prevalence of new parasitic infection, especially with *Giardia* spp., was significantly decreased with vitamin A intervention, suggesting an immune regulatory modulation of this nutrient on parasitic intestinal infections.

Acknowledgments

The intervention study was supported by NIAID ICIDR Grant No.UO1-AI-026512 from the National Institutes of Health in Bethesda, MD and part by Brazilian funding agency, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Ministério de Ciência e Tecnologia, Brasília, Brazil. RLG and AAML were responsible for the development of the original proposal, study protocol, follow-up, quality control, collection and validation of the data. AMS and RMSM were responsible for the database and analysis of the data. NLL was responsible for the clinical

follow up of the children and adverse event system. AAML was responsible for the lactulose and mannitol measurements, provision of advice and consultation. WSB was responsible for the retinol, serum concentration measurements. BLLM provided nutritional consultation and help to write the manuscript. All authors participate in the data analysis, writing and interpretation of the manuscript. The authors did not have financial or personal relationships with the organization sponsoring the research during the time of the study.

Financial support: Brazilian funding agency, CNPq, and ICIDR program Grant # 5 U01 AI026512 from NIH, USA supported this study.

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Figure 1.
Flow diagram for the population and the protocol activities during the intervention study.

Figure 2.

Repeat measurements of intestinal barrier function parameter in the same child by time and groups during four months follow-up in children participating in the intervention study at urban community Parque Universitário, Fortaleza, CE, Brazil, June, 2000 to October, 2007.

Figure 3.

Incidence of total intestinal parasitic and *Giardia* spp. at enrollment and one month follow-up in children participating in the vitamin A treatment study at urban community Parque Universitário, Fortaleza, CE, Brazil, June, 2000 to October, 2007. Stool samples were available for parasitic examination on 85% (63/74; 33 in the placebo and 30 in the vitamin A group) at enrollment and 79% (56/71; 31 in placebo and 25 in the vitamin A group).

Table 1

Characteristics of the children participating in the intervention study by age, sex, nutritional status, lactulose:mannitol ratio and serum retinol in Parque Universitário, Fortaleza, CE, Brazil.

Parameters	Total N = 74	Retinol * N = 36	Placebo N = 38
Age (months; mean $\pm$ SD)	43.3 $\pm$ 27.72	40.8 $\pm$ 27.24	45.6 $\pm$ 28.32
Sex N (%)			
Male	42 (57)	22 (61)	20 (53)
Nutritional z-scores (mean $\pm$ SD)			
Height-for-age	-1.138 $\pm$ 0.883	-0.988 $\pm$ 0.806	-1.280 $\pm$ 0.939
Weight-for-age	-0.742 $\pm$ 1.033	-0.626 $\pm$ 0.968	-0.852 $\pm$ 1.093
Weight-for-height	0.000 $\pm$ 1.010	-0.048 $\pm$ 0.961	-0.046 $\pm$ 1.228
Serum retinol (μ M; mean $\pm$ SD)	1.25 $\pm$ 0.266	1.25 $\pm$ 0.258	1.25 $\pm$ 0.286
Fecal lactoferrin N (%)	14/60 (23)	4/29 (14)	10/31 (32)
Lactulose:mannitol ratio median (range)	0.0890 (1.125)	0.0690 (0.70)	0.0890 (1.13)

* Retinol group = retinyl palmitate (100,000 IU for children <12 months and 200,000 IU for children at least 12 months old); or placebo = tocopherol 40 IU, treated each four months dose at 0, 4 and 8 months of the study protocol.

The Student's *t* tests were used to compare means on normally distributed and continuous variables and the parameters that did not follow the normal distribution were analyzed using Mann-Whitney tests, Chi-square tests or Fisher exact tests. There were not significantly difference ($p > 0.05$) when compared these parameters between placebo versus vitamin A groups.

Table 2

Cumulative anthropometric z-scores by time and groups adjusted for age and season during twelve months follow-up in children participating in the intervention study at urban community Parque Universitário, Fortaleza, CE, Brazil.

Cumulative nutritional z-scores [*]	Total	Retinol [‡]	Placebo	P [§] Values
Weight-for-height				
0 – 4 months (N=66)	0.2094 ± 0.0873	0.2642 ± 0.1206	0.1609 ± 0.1263	0.3609
0 – 8 months (N=59)	0.0929 ± 0.1021	0.2720 ± 0.1392	−0.0388 ± 0.1421	0.1152
0 – 12 months (N=55)	0.1598 ± 0.1137	0.3009 ± 0.1349	0.0584 ± 0.1691	0.2539
0 – 24 months (N=47)	0.2638 ± 0.1738	0.4658 ± 0.1616	0.1268 ± 0.2699	0.3827
0 – 36 months (N=40)	0.2612 ± 0.1913	0.4159 ± 0.2604	0.1470 ± 0.2737	0.5968
Weight-for-age				
0 – 4 months (N=66)	0.1130 ± 0.0492	0.1561 ± 0.0630	0.0749 ± 0.0744	0.2196
0 – 8 months (N=59)	0.0659 ± 0.0640	0.1100 ± 0.0969	0.0335 ± 0.0860	0.7296
0 – 12 months (N=55)	0.0913 ± 0.0751	0.0922 ± 0.0967	0.0906 ± 0.1067	0.8802
0 – 24 months (N=53)	−0.0296 ± 0.1108	0.0082 ± 0.1150	−0.0565 ± 0.1725	0.9905
0 – 36 months (N=52)	−0.1369 ± 0.1150	−0.1438 ± 0.1772	−0.1323 ± 0.1533	0.7204
Height-for-age				
0 – 4 months (N=66)	−0.0982 ± 0.0553	−0.1197 ± 0.0902	−0.0791 ± 0.0682	0.8024
0 – 8 months (N=59)	−0.0129 ± 0.0738	−0.1472 ± 0.0927	0.0859 ± 0.1063	0.0499
0 – 12 months (N=55)	−0.0551 ± 0.0840	−0.2526 ± 0.1255	0.0869 ± 0.1074	0.0236
0 – 24 months (N=53)	−0.3177 ± 0.1079	−0.4259 ± 0.2110	−0.2410 ± 0.1093	0.2895
0 – 36 months (N=52)	−0.3679 ± 0.1423	−0.5657 ± 0.2766	−0.2339 ± 0.1472	0.2473

* Data are shown as mean ± standard error for cumulative changes (final minus initial measurement for the 12 months follow-up period) in anthropometric z-scores.

[‡] Retinol group = retinyl palmitate (100,000 IU for children <12 months and 200,000 IU for children at least 12 months old); or placebo = tocopherol 40 IU, treated each four months dose at 0, 4 and 8 months of the study protocol.

[§] Covariance analysis (ANCOVA) was used to correct the influence of age and seasonality when compared to anthropometrics parameters. The statistically significant difference was accepted when the alpha value was 0.05 or less.