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Vitamin A Deficiency Alters Airway Resistance in Children With Acute Upper Respiratory Infection

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Summary. Objective: To assess whether vitamin A deficiency alters the recovery of total respiratory resistance (TRR) following acute upper respiratory tract infection (URI). Methods: This is a case control study of children, age 4–6 years and grouped as: URI, (n = 74), URI and wheezing, (URI-wheezing, n = 52), and healthy controls (n = 51). Vitamin A and total respiratory resistance (TRR) were assessed using the modified relative dose response (MRDR) and forced oscillometry, respectively. Results: Children with URI and URI-wheezing had lower retinol, 32.4 ± 13.12 and 18.3 ± 6.83 μg/dl respectively, compared to controls, 56.9 ± 29.82 μg/dl (ANOVA, $P < 0.001$). The MRDR was elevated in children in the URI or URI-wheezing groups 0.066 ± 0.045 and 0.021 ± 0.021, respectively, compared to controls 0.007 ± 0.006 (ANOVA, $P < 0.0001$). The TRR in the URI and URI-wheezing groups differed from controls. During convalescence, the TRR failed to decline in the URI-group only when the MRDR was >0.06. In the URI-wheezing group, TRR declined independently of retinol and MRDR. Conclusion: Vitamin A contributes to preservation of airway function during and in recovery after upper respiratory infection in children. **Pediatr Pulmonol. 2013; 48:481–489.**

Key words: Asthma; modified relative dose response test; forced oscillation; wheezing; respiratory virus.

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INTRODUCTION

Respiratory infections by viruses such as parainfluenza, respiratory syncytial virus (RSV), influenza, coronavirus, or rhinovirus can trigger acute episodes of asthma.[1] Viral infection causes inflammation of the lower respiratory tract in both atopic and nonatopic individuals, but this usually only becomes clinically significant in individuals with pre-existing asthma. Affected individuals exhibit decreased forced expiratory volume in 1 sec (FEV1) and increased bronchial hyperreactivity,[2] which normalize after the infection. When infection is coupled with deficiency of micronutrients such as iron, zinc or vitamin A, recovery may be modified.[3]

Plasma retinol may diminish from reduced intake, or decreased absorption. Vitamin A deficiency (VAD) can cause metaplasia with keratinization of the tracheal and bronchial epithelium.[4,5] Prior studies in children suggest

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that lower vitamin A intake is associated with lower conducting airflow. Kassaye et al.[6] showed that 40.6% of children who had low vitamin A reserves had a mean FEV1 that was 48.8 ml lower than the reference group. Gilliland et al.[7] found that lung function was lower in children whose diet included fewer fruits, vegetables, and vitamin A, C, and E. Checkley and associates demonstrated that prenatal maternal supplementation with retinol improved lung function in their offspring.[8] A meta-analysis of vitamin A supplementation identified nine clinical trials in children aged 6 months to 7 years old, and only two studies demonstrated that vitamin A supplementation protected against the lower respiratory tract infection.[9] Other studies showed an adverse effect of vitamin A supplementation on respiratory tract infection.[10] A more recent meta-analysis showed that lower vitamin A intake was associated with increased odds of asthma and wheezing.[11]

There are conflicting results from studies in rodents. McGowan et al.[12] showed that VAD increased airway responsiveness in rats with lower M2 muscarinic receptor function, which may also contribute to asthma.[13] In contrast Shuster et al.[14] showed that VAD protected against bronchial hyperreactivity associated with eosinophilic inflammation after antigen challenge in mice. Because mechanistic studies in rodents suggest that VAD may alter more than one pathway, they have limited predictive value in humans.

Although airway development and function may be influenced by the level of vitamin A intake, it is unclear whether this alters the course of respiratory illnesses or has long term clinical consequences. We hypothesized (a) that adequate vitamin A is required for airway epithelial repair after injury and (b) that the effects of VAD may be more readily discerned in an acute illness which primarily involves the conducting airways (rather than the alveolar region as in pneumonia). To address this hypothesis we studied children age 3 through 7 in a region of Brazil where children are at risk for VAD. The primary outcomes were the level of total respiratory resistance (TRR) during and its normalization after recovery from an acute upper respiratory illness. Serum retinol and the modified relative dose response (MRDR) were determined during the acute illness, to assess the relationship between vitamin A status and changes in pulmonary function during an upper respiratory infection). We found that children with lower vitamin A had a lag in normalization of airway resistance after an upper respiratory infection (URI).

METHODS

Enrollment

Using a cross sectional study design, 180 children, age 4 through 6 years were enrolled from outpatient clinics and schools in Natal, RN, Brazil between September 2007 to March 2010. Two pediatric pulmonologists enrolled all of the subjects, administered the questionnaire, and confirmed that those enrolled met all of the enrollment criteria. The children comprised three groups: (a) acute upper respiratory infection (URI, $n = 74$), the URI children had symptoms of sore throat and or pharyngeal erythema within 7 days prior to enrollment and were without signs of pneumonia or wheezing on physical examination; (b) upper respiratory infections and wheezing (URI-wheezing, $n = 52$) children with symptoms of URI and wheezing on presentation; and (c) healthy controls, who had no respiratory symptoms for 4 weeks prior to enrollment ($n = 51$). Controls were children who came to the clinic with their parent or guardian, accompanying another child who was seeking medical assistance. The control children did not have any immediate health complaints and had no symptoms of a respiratory illness during the last four weeks. The required number of subjects per group was estimated, based on the prevalence of sub-clinical vitamin A deficiency in this population and on predicted changes in the total respiratory resistance (TRR). The original estimate predicted that 65 cases and controls were required to achieve a power of 0.8 and an alpha of 0.05. We ultimately enrolled 51 controls and 74 URI-without wheezing patients. After most of the patients in these groups had been enrolled, it became apparent that very few URI-without wheezing patients had a TRR $> 150\%$ predicted. Therefore a third group was enrolled, comprised of 52 children who had wheezing at initial presentation with a URI and whose TRR was significantly elevated. A questionnaire addressing respiratory health, history of atopy,[15] home environment was completed by the caregiver of the children. The subject and their accompanying caregiver were asked to report what the child had eaten for each meal during the prior 24 hr, and specifically questioned whether they consumed foodstuffs that contain retinol or carotenoids (milk, margarine, carrots, a particular squash available in Northeast Brazil, eggs, fruit juice, meat, and green leafy vegetables).[16] Anthropometric measurements were obtained and a complete physical examination was performed. The analog balance and stadiometer that were used to assess weight and height were produced by Filizola Co. (Brazil) and were graduated in 100 g and 1 mm, respectively. The children were weighed by a trained nursing assistant and their clothing was not removed prior to weighing. Children with a diagnosis of pneumonia (based on rales, fever and/or chest radiograph, vitamin supplementation within the prior 6 months, or oxygen saturation $<92\%$ were excluded. This protocol and consent documents were reviewed and approved by the Human Subjects Committee at the University of Iowa (the awarded

institution), the Universidade Federal do Rio Grande do Norte Ethical Committee (CEP-UFRN 96-03) and by the Brazilian National Ethical Committee (CONEP 808-2005). Guardians of the participating children provided consent on behalf of the participants.

Evaluation of Vitamin A Status

The Modified Relative Dose Response test (MRDR) was administered by giving 1.5 mg of 3.4 didehydroretinol acetate (DRA), an analogue of vitamin A, orally followed by ingestion of cashew nuts (6 units) to maximize absorption of the DRA.[17] Four hours after of ingestion, venous blood was collected in a citrated tube. The blood was kept on ice, protected from ambient light, and serum was frozen at -80°. Five hundred microliters of serum were supplemented with retinol acetate as an internal standard. After deproteination using ethanol, the serum was extracted twice with 0.75 ml of hexane. The extract was dried under a stream of nitrogen in the dark. After dissolving the lipid extract in ethanol, retinol, didehydroretinol and retinol acetate were resolved using isocratic HPLC.[18] Retinol and didehydroretinol standards were prepared from retinol acetate and DRA, respectively and used to calibrate each run. A cutoff of MRDR of >0.060 children identified with low reserves of vitamin A deficiency (VAD subclinical), a serum retinol <10 μg/dl and from 10 to 20 μg/dl retinol defined clinical and subclinical VAD, respectively.[17] Blood was not obtained during the return visit.

A portion of the serum was used to assess retinol binding protein 4 (RBP) using a sandwich ELISA (Immunology Consultants Laboratory, Newberg, OR), following the manufacturer's instructions. Additional portions of the serum were used to assess the hemogram, total protein, albumin, transferrin, and C-reactive protein in the certified Department of Clinical Analysis laboratory at UFRN, Natal, RN, Brazil.[19]

Evaluation of Total Respiratory Resistance (TRR)

Total respiratory resistance was measured by trained pediatric pulmonolgists during quiet tidal breathing using forced oscillation (FOS) with an impulse oscillometer (Masterscreen IOS, Erich Jaeger, Hoechberg, Germany) following American Thoracic Society guidelines.[20] The child's cheeks and sub-mandibular region were supported during the measurements. A minimum of three reproducible measurements were performed for each child at each measurement session. The oscillometric measurements were performed at a single session for controls and twice in the URI and URI-wheezing groups (upon initial enrollment and at a return visit after 3 weeks).

Statistical Analyses

Categorical variables were analyzed using the chi-square (χ^2) test. The normality of quantitative variables was assessed using the Kolmogorov–Smirnov test. Variables with normal distribution were presented as means and standard deviations. Student's *t*-test or analysis of variance (ANOVA) was used to investigate means between groups. Results were considered significant for P values <0.05. Analysis was performed using the Statistical Package for Social Sciences, version 11.5 (SPSS Inc., Chicago, IL).

RESULTS

Anthropometric Characteristics

A total of 177 children were examined. The mean age of the control group was slightly but significantly older than the URI group (60.6 vs. 57.4 months ANOVA $P = 0.04$; Table 1). There were no gender differences among the three groups (χ^2, $P = 0.24$; Table 1). The anthropometric data (Table 2) showed no significant differences among the groups. Only 8 of the 126 (6.4%) children who were scheduled to return in 3 weeks failed to complete the second set of FOS measurements.

Vitamin A Status During URI

The mean serum retinol in the control group was 56.78 ± 29.82 μg/dl. Children with URI-wheezing had a significantly mean lower serum retinol (18.29 ± 6.83 μg/dl, $P < 0.001$), compared to other groups (Table 3). The MRDR for children in the URI or

TABLE 1— Demographic Composition of the Study Groups

	Total (n = 177)	URI (n = 74)	URI-wheezing (n = 52)	Control (n = 51)	*P* value
Age (months; mean ± SD)	59.0 ± 1.58	57.4 ± 6.95	59.0 ± 6.80	60.6 ± 6.41	0.037*
Gender n (%)					
Male	89 (50.3)	38 (51.4)	30 (57.7)	21 (41.2)	0.238**
Female	88 (49.7)	36 (48.6)	22 (42.3)	30 (58.8)	

*One-way ANOVA.
**χ^2.

TABLE 2— Anthropometric Characteristics of the Groups

Z-scores—mean ± SD	Control (n = 51)	URI (n = 74)	URI-wheezing (n = 52)	Total (n = 177)	P^* value ANOVA
Height/Age	−0.21 ± 0.88	−0.43 ± 1.02	−0.15 ± 0.89	−0.29 ± 0.95	0.216
Weight/Age	0.34 ± 1.09	0.09 ± 1.14	0.29 ± 1.18	0.22 ± 1.14	0.474
Weight/Height	0.67 ± 1.87	0.54 ± 1.32	0.50 ± 1.18	0.56 ± 1.42	0.899

Mean ± SD.
*Of the 177 children, a total of 101 (24 controls, 47 URI and 30 URI-wheezing) were used to calculate the ratio of weight (kg) per height (cm), according to the World Health Organization tables for calculating *Z*-scores (WHO 2006) only children with height up to 121 cm should be used.

URI-wheezing groups, 0.066 ± 0.045 and 0.021 ± 0.021, respectively, differed significantly from the control group, (0.007 ± 0.006, $P < 0.001$, Table 3). Fifty-five percent of the children had retinol ≤30 μg/dl, Table 3. To evaluate correlations with the TRR, retinol and the MRDR were stratified as categorical variables. Comparison of these categories showed that the lower levels of retinol were predominantly observed in the URI and URI-wheezing groups (χ^2, $P < 0.001$). The highest mean MRDR was observed in children with URI (χ^2, $P < 0.$ 001), Table 3. The percentages of URI-wheezing children who had consumed milk or meat during the prior 24 hr were significantly lower than the control and URI groups, $P = 0.003$ and $P = 0.000$, respectively (Pearson χ^2 coefficient). Consumption of all of the other foodstuffs was similar among the three subject groups.

We queried whether acute inflammation or infection, which may be accompanied by a higher level of C-reactive protein, could explain the lower retinol in the URI-wheezing group or the elevated MRDR in the URI group. The C reactive protein in children with URI and URI-wheezing, 3.0 ± 3.1 and 3.0 ± 3.0 mg/L, respectively, did not differ from controls (ANOVA, $P > 0.05$, Table 4). In all groups, the mean CRP was <5 mg/L, considered by some as the lower limit for inflammation.[21] We examined the relationship between CRP and the MRDR, as continuous variables using linear regression, and found no significant correlations when each group was considered individually or when all groups were combined (data not shown). Serum retinol binding protein 4 (RBP), an inverse acute phase reactant, was also similar among the three groups. All groups had normal serum albumin with a combined mean of 3.7 ± 0.17 g/dl although there was a significant difference between URI-wheezing and control (ANOVA, $P = 0.020$). There was a significant positive linear correlation between albumin and RBP only in the URI-wheezing group ($P = 0.023$, R = 0.330). Transferrin and iron were similar among all three groups of subjects (Table 4). The arithmetic means for the absolute number of peripheral eosinophils did not differ among the three groups (not shown).

Respiratory Resistance Is Increased in Children With URI and Vitamin A Deficiency

During the initial encounter, the mean percent predicted TRR (R5Hz) was increased in the URI-wheezing group with R5Hz of 126.0 ± 26.9 ($P = 0.001$),

TABLE 3— Vitamin A and MRDR Status

Levels of vitamin A	Total (n = 153), n (%)	Control (n = 38), n (%)	URI (n = 69), n (%)	URI-wheezing (n = 46), n (%)	P^* value
Group mean retinol (μg/dl)	35.81 ± 19.47	56.78 ± 29.82	32.37 ± 13.12	18.29 ± 6.83*	0.000†
Retinol > 30 μg/dl	69 (45.1)	30 (78.9)	36 (52.2)	3 (6.5)	
Retinol 20–30 μg/dl	41 (26.8)	7 (18.4)	20 (29.0)	14 (30.4)	0.001††
Retinol 10–20 μg/dl	37 (24.2)	1 (2.6)	12 (17.4)	24 (52.2)	
Retinol < 10 μg/dl	6 (3.9)	0 (0.0)	1 (1.4)	5 (10.9)	
Group mean MRDR	0.031 ± 0.030	0.007 ± 0.006	0.066 ± 0.045**	0.021 ± 0.021*	0.000†
MRDR <0.030	90 (59.0)	38 (100.0)	20 (29.0)	32 (69.6)	
MRDR 0.030–0.060	22 (14.2)	0 (0.0)	9 (13.0)	13 (28.3)	0.001††
MRDR >0.060	41 (26.8)	0 (0.0)	40 (58.0)	1 (2.2)	

Mean ± SD.
†1-way ANOVA for differences across all subject and retinol groups.
*$P < 0001$ compared to control and URI without wheezing using 1-way ANOVA.
**$P < 0.001$ compared to control and URI with wheezing using 1-way ANOVA.
††χ^2 test for differences across all subject and retinol groups.

TABLE 4— Serum Constituents That Can Be Altered by Inflammatiion or Infection

Parameters	Total $P^{\dagger}$ (n = 153), mean ± SD	Control (n = 38), mean ± SD	URI (n = 69), mean ± SD	URI-wheezing sibilância (n = 46), mean ± SD	*P* value ANOVA
C Reactive protein (mg/L)	2.7 ± 0.5	2.1 ± 0.7	3.0 ± 3.1	3.0 ± 3.0	0.172
Albumin‡ (g/dl)	3.7 ± 0.17	3.9 ± 0.4	3.8 ± 0.5	3.5 ± 0.7	0.020
Transferrin (mg/dl)	234.3 ± 41.7	237.0 ± 34.1	224.1 ± 39.2	241.9 ± 53.0	0.059
Transferrin saturation (%)	30.4 ± 17.2	31.5 ± 18.5	31.3 ± 18.5	25.9 ± 13.1	0.062
Iron (μg/dl)	55.9 ± 7.7	60.4 ± 37.8	51.4 ± 42.0	56.0 ± 52.9	0.571
Retinol binding protein 4 (μg/ml)	19.6 ± 9.8	21.3 ± 11.0	19.8 ± 10.4	17.7 ± 6,7	0.203

Mean ± SD.
[†]Post-hoc test showed a difference in the level of albumin between the control group and the group with URI-wheezing.

whereas the URI group was similar to the control group whose mean was 93.6 ± 18.0% predicted, Table 5. The regression equation for control children, R5Hz = 3.127–0.0188(height), is similar to another population of children.[22] Total respiratory resistance decreased during recovery from URI symptoms, although the decrease was slight in the group without wheezing (Table 5).

The recovery of airway function was assessed by comparing the TRR during and after a symptomatic URI for each subject in the URI and URI-wheezing cohorts. The group-mean TRR did not change significantly for children with URI, without wheezing. However there was a significant inverse linear relationship between the MRDR or retinol and the diminution in TRR for individuals in the URI-group (Fig. 1). A similar relationship was not observed for the MRDR or retinol in the URI wheezing group. During recovery, the children with wheezing showed a larger decrement in TRR than occurred in children without wheezing. The subjects in the URI group without wheezing, whose MRDR was >0.06, did not show a reduction in TRR after resolution of symptoms, whereas subjects whose MRDR was <0.06 had a significant reduction in their TRR after their symptoms resolved (Table 6).

DISCUSSION

Children at risk for marginal vitamin A deficiency, within the URI-only and URI-wheezing groups exhibited a more elevated MRDR (suggesting that their liver stores may be reduced), or lower circulating retinol, respectively during an acute upper respiratory illness. An elevated MRDR correlated with an attenuated normalization of airway resistance during the recovery from the upper respiratory illness in children without wheezing.

Retinol may decline during an acute phase response and return to normal after resolution of infection, trauma or inflammation without an alteration in vitamin A body stores.[23] Others found lower levels of serum retinol in 15% of children admitted with pneumonia but after convalescence, 77% of these children had regained normal levels.[24] We observed that 63% of the children in the URI-wheezing group had serum retinol <20 μg/dl whereas the MRDR was >0.060 in only 2.2% of these children. In the URI group only 18.8% had retinol <20 μg/dl, but the MRDR exceeded 0.06 in 58% of the subjects, indicating low liver reserves. The MRDR and retinol were both normal in the control group. Observing a group mean MRDR in a vitamin A-sufficient

TABLE 5— Respiratory Resistance (R5Hz) of the Groups During Symptoms and 3 Weeks Post-Respiratory Symptoms

	R5Hz						
	Initial** (mean ± SD)			Recovery (mean ± SD)			
Groups	n	kPa/(L/s)	%	n	kPa/(L/s)	%	P^{*} value
Control	51	1.08 ± 0.21	93.6 ± 18.0	—		—	—
URI	74	1.15 ± 0.20	97.2 ± 16.6	72	1.13 ± 0.19	93.6 ± 16.0	0.022
URI-wheezing	52	1.47 ± 0.32	126.0 ± 26.9	51	1.14 ± 0.26	96.8 ± 21.5	0.000

Mean ± SD.
[*]Two-way repeated measure ANOVA on ranks comparing the initial and recovery TRR for individual subjects. %, percent predicted.
[**]Significant difference in the initial R5Hz between URI and URI-wheezing by the Post-hoc test, ($P = 0.0000$); but no difference between URI and controls ($P > 0.05$).

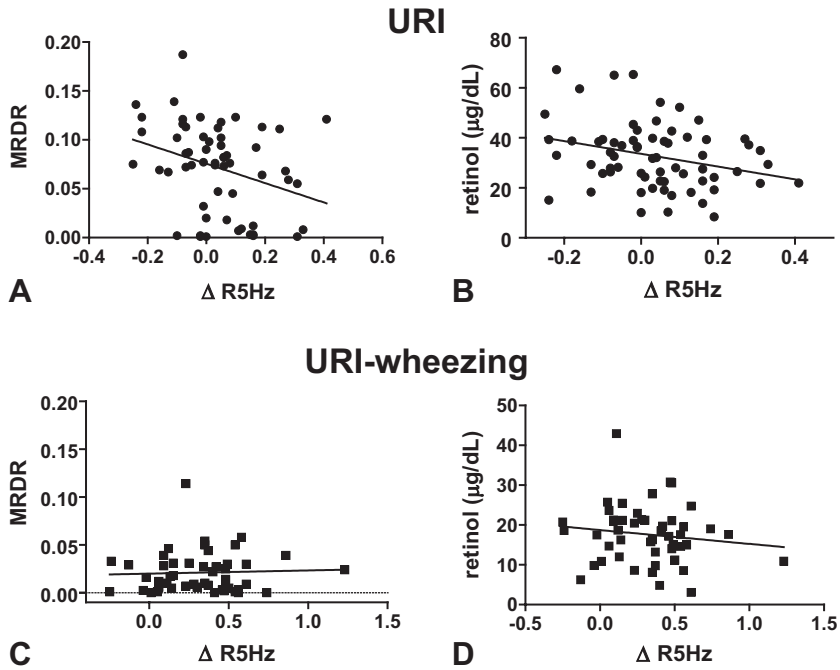

Fig. 1. Decrease in TRR is inversely related to vitamin A stores during URI. The second measurement of TRR (3 weeks after initial) was subtracted from the initial measurement (during acute symptoms of URI) to calculate the change in R5Hz in kPa/(L/s) (Δ R5Hz) for each subject. Negative indicates increase in TRR. MRDR is ratio of serum 3,4 didehydroretinol to retinol. Line is linear regression fit. For URI-group, (A) R = 0.301 general linear model *P* = 0.015. B: R = 0.29 general linear model *P* = 0.02 (C) and (D) URI-wheezing, slopes of regression lines did not differ significantly from zero.

range but mean serum retinol in a deficient may represent a decline in retinol related to the current URI. We observed a weak, although significant, inverse linear relationship between RBP and CRP when the URI and URI-wheezing groups were combined ($P = 0.0285$, $R = 0.27$). This suggests that the lower retinol in these groups correlates with lower availability of its serum carrier protein (RBP). There was a significant positive correlation between albumin and RBP ($P = 0.023$, $R = 0.331$) in the URI-wheezing group, suggesting that these subjects had a decline in a second liver-derived protein, in addition to RBP. Therefore diminished synthesis may have contributed to the lower RBP in the URI-wheezing group. Others have shown that serum retinol may decline because of a short-term increase in renal clearance (even if the CRP remains <5 mg/L), without depletion of the larger retinyl ester storage pool in the liver.[25] The percentage saturation of transferrin

TABLE 6— Smaller Decrement in TRR After Recovery of Symptoms Correlates With Lower Higher MRDR in Children With URI, but Without Wheezing

	TRR (R5Hz*,†)								
	Symptomatic phase				Post-recovery				
MRDR	n	% subjects	TRR (mean ± SD)	% predicted TRR	n	% subjects	TRR (mean ± SD)	% predicted TRR	*P*-value
>0.060	40	58.0	1.14 ± 0.22	96.0 ± 18.2	39	58.2	1.15 ± 0.21	96.3 ± 16.9	0.565
0.030–0.060	9	13.0	1.17 ± 0.16	98.8 ± 11.0	9	13.4	1.04 ± 0.09	86.5 ± 6.2	0.020
<0.030	20	29.0	1.15 ± 0.17	99.1 ± 16.0	19	28.3	1.05 ± 0.16	88.2 ± 15.0	0.003
Total	69	100.0			67	100.0			

Mean ± SD.
*Two-way repeated measure ANOVA on ranks showed a significant difference between the symptomatic and post-recovery measurements when the MRDR was between 0.030–0.060 and <0.03.
†Mean ± SEM.

with iron showed a trend towards being lower in the URI-wheezing group, although the iron content did not differ from the control and URI-groups.

We used the MRDR so estimate hepatic vitamin A stores. The MRDR is most commonly expressed as a categorical variable, with 0.059 as an upper limit of normal. Those with a MRDR $\geq$ 0.06 are considered to be marginally vitamin A deficient, although their serum retinol is in the normal range. Others have sub-divided the normal range and correlated other variables with the sub-divisions; although they have lower predictive value for health consequences than the traditional cut-off of 0.059.[17,26] The MRDR has some weaknesses as a surrogate of hepatic vitamin A stores, because like retinol the newly acquired serum 3,4 DHR is also bound to RBP, which may diminish during inflammation.[27] Using the isotope dilution technique may circumvent this weakness.[28]

Prior comparisons of serum retinol in children with and without asthma have varied. Some have observed a correlation between low serum retinol and the severity of wheezing[29,30] or history of asthma.[31,32] Our observation of a low serum retinol during URI with wheezing in children with a prior history of asthma is consistent with most prior studies. The overall mean MRDR for the URI-wheezing group was similar to controls, but more children in the URI-wheezing group had retinol between 10 and 20 μg/dl than in the other two groups. In children hospitalized for pneumonia, Stephensen and associates observed that a smaller percentage of children with a CRP exceeding 10 mg/L exhibited lower estimated liver stores (by the relative dose response test) than did children with CRP $<$10 mg/L.[33] Thus others have encountered disparities between liver stores and circulating retinol during a more severe respiratory illness.

It is more difficult to explain why the children in the URI group had a higher mean MRDR, suggesting that their estimated liver stores were reduced, whereas their mean serum retinol was normal. A less severe acute illness may account for the observation of a normal mean retinol. Fifty-eight percent of the children without wheezing had a MRDR $>$ 0.06 during the URI, whereas this was observed in only one child with wheezing, and in none of the controls. As an assessment of liver stores, the MRDR is a time-average of vitamin A intake over months rather than weeks or days. Only two children in the URI-group and two in the URI-wheezing group had symptoms for more than 7 days, making it unlikely that liver stores diminished during the acute illness. Therefore, estimated liver stores in URI-children were chronically lower than in the URI-wheezing and control groups. Because this was unanticipated, we evaluated several potential explanations. No children were supplemented with vitamin A during the 6 months prior to enrollment, and 29.4% and 16.0% of children in the control and URI-groups, respectively, had received vitamin A between 6 and 12 months prior to enrollment. Differences in diet, not discerned by the 24-hr recall may have contributed. Most of the children in the URI- and URI-wheezing groups were enrolled during the rainy season (April through August). However the peak enrollment for the control group was during January through March. Seasonal vitamin A-rich fruits and vegetables may have been more readily available during October through January, just prior to enrollment of the majority of the control children. We also assessed the correlation between the MRDR and absolute blood eosinophil count. A larger fraction of the children in the URI group had eosinophils at the high end of the range compared to the other two groups (see E-Figure 1). This suggests that intestinal helminthes may have been more prevalent in children of the URI group. Intestinal helminthes deplete hepatic vitamin A stores, resulting in increased MRDR which can be normalized by deworming.[34,35]

Others have used forced oscillation (FOS) to estimate TRR in children. Delacourt and associates evaluated respiratory mechanics in asthmatic children and found that FOS correlated with spirometry.[36] Because FOS does not require a maximal expiratory effort, it was more suitable in children between 3 and 7 years of age, when VAD is most common. Similar to others, we observed a coefficient of variation (CV) for the combined R5Hz (TRR) measurements in all subjects of 9%.[37] The mean change in TRR between the first and second encounter was 34% for the URI-wheezing and 19% for the URI groups respectively, which is greater than the CV of the within the same day measurements. Therefore, although the observed changes were small, they cannot be attributed to inadequate reproducibility.

We hypothesized that vitamin A status may influence the decrease in TRR that would be expected during recovery from a viral URI. Although TRR remained within the normal range for all children with URI and without wheezing, it decreased significantly during recovery only in children in the URI group whose MRDR was $<$0.06. In the URI-group, children with the most elevated MRDR had the smallest decline in TRR during convalescence (Table 6). However, within the URI-wheezing group, the convalescent decline in TRR was not less in those children with lower serum retinol. The lower retinol in the URI-wheezing group may result from an inflammatory response. Children in the URI-wheezing group with the highest MRDR did not show a diminished normalization of TRR after recovery from their URI symptoms. Two factors may contribute to this variance from the group of children with URI but without wheezing. First, there was only one child in the URI-wheezing cohort whose MRDR exceeded 0.06.

And second, the convalescent incremental decrease in TRR was much greater in the URI-wheezing group. This suggests that in this group, in which nearly all children had a prior history of asthma, other contributors to airway inflammation and wheezing, may have obscured a more subtle effect of vitamin A. An alternate interpretation of our findings is that the lower mean MRDR (and presumably more abundant vitamin A reserves) in the URI-wheezing compared to the URI group predisposes the children to wheezing, as others observed in mice, which were sensitized to ovalbumin.[14] Although airway eosinophils increase in asthmatics during a URI, neutrophils and Th1 T-cells also increase, which differs from the ovalbumin model in mice where changes are almost exclusively observed in airway eosinophils.[38,39]

The mechanisms behind this association between reduced vitamin A and delayed normalization of TRR remain undefined. This case–control study yielded correlative data, and cannot conclusively define mechanisms. However our findings may be interpreted in light of recent findings about how retinoids influence the airway immune response. Allergic, Th2-driven inflammation is a hallmark of asthma and bronchial hyperreactivity. Studies in mice suggest that retinoic acid (RA, an important biologically active vitamin A-metabolite) establishes and maintains forkhead box (Fox)P3+ regulatory T-cells (Treg).[40] Along with transforming growth factor β and rapamycin, RA can reprogram Th2 cells into FoxP3+ Tregs, and reduce airway inflammation. A recent study of Ecuadorian children showed that circulating FoxP3+ Tregs diminish prior to age 5 years as differentiated CD4 and CD8 T-cells increase.[41] Poor vitamin A nutriture during the first 5 years of life may enhance the loss of Tregs and afford reduced protection against airway reactivity.

It is unclear whether the lack of decrease in TRR in children with MRDR > 0.06 has physiological or symptomatic consequences. Checkley et al.[42] found that children receiving vitamin A supplementation during pregnancy or during the their pre-school years did not show a reduced prevalence of wheezing or asthma when they reached the ages of 14 through 23 years when lung development has ceased. Further longitudinal studies are required to assess whether vitamin A supplementation alters airway reactivity while the lungs are still developing (prior to cessation of the age-related decrease in airway resistance).[43]

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