ERJ Express. Published on June 23, 2011 as doi: 10.1183/09031936.00006911

Supplementation with vitamin A early in life and subsequent risk of asthma

William Checkley MD, PhD (1, 2), Keith P West Jr DrPH (3), Robert A Wise MD (1), Lee Wu MHS (3), Steven C LeClerq MPH (3, 4), Subarna Khatry MD (4), Joanne Katz ScD (2), Parul Christian DrPH (3), James M Tielsch PhD (2), Alfred Sommer MD, MHS (5).

1. Division of Pulmonary and Critical Care, School of Medicine, Johns Hopkins University, Baltimore, MD.
2. Program in Global Disease Epidemiology and Control, Department of International Health, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD.
3. Center for Human Nutrition, Department of International Health, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD.
4. The Nepal Nutrition Intervention Project Sarlahi (NNIPS), National Society for the Prevention of Blindness, Kathmandu, Nepal.
5. Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD.

Correspondence:

William Checkley, MD, PhD

Assistant Professor

Division of Pulmonary and Critical Care

Department of Medicine

Johns Hopkins University

Baltimore, MD 21212

Telephone: 443-287-8741

Email: wcheckl@jhmi.edu

Word count: 3,210.

Running title: Vitamin A and risk of asthma.

Keywords: asthma, vitamin A, longitudinal study, randomized clinical trial.

Role of funding source: This work was carried out by the Center for Human Nutrition, Department of International Health of the Johns Hopkins Bloomberg School of Public Health (Baltimore, MD) and the Division of Pulmonary and Critical Care of the Johns Hopkins University, School of Medicine, The Johns Hopkins University (Baltimore, MD) through the Nepal Nutrition Intervention Project, Sarlahi in collaboration with the National Society for Comprehensive Eye Care (Kathmandu, Nepal). This study was funded by the Bill and Melinda Gates Foundation grant number 614 (Seattle, WA) and the Sight and Life Research Institute (Baltimore, MD). The original maternal vitamin A or beta-carotene supplementation trial (1994-1997) was conducted under the Vitamin A for Health Cooperative Agreement number HRN-A-00-97-00015-00 between the Johns Hopkins University and the Office of Health,

Infectious Diseases and Nutrition of the United States Agency for International Development (Washington, D.C.), with additional support from Task Force Sight and Life (Basel, Switzerland). William Checkley was further supported by a Clinician Scientist Award from the Johns Hopkins University and a Pathway to Independence Award from the National Heart, Lung and Blood Institutes (K99HL096955). We especially thank Sharada Ram Shrestha (deceased) for his dedicated contributions to the field study. The sponsors had no role in the study design, writing of the report or decision to submit the paper for publication.

Authors contributions: All authors participated in the study design and data collection of either the original or follow-up studies and contributed to data interpretation and writing of the manuscript. William Checkley is responsible for the literature search, development of figures and tables, conduct (and replication) of data analysis, data interpretation and writing of the first draft of the manuscript and subsequent revisions. Ms. Lee was equally responsible for the conduct (and replication) of data analysis.

Disclosures: The authors have no financial relationships relevant to this article to disclose.

Abstract

Background: Animal models suggest that vitamin A deficiency affects lung development adversely and promotes airway hyper-responsiveness, and may predispose to an increased risk of asthma. We examined the long-term effects of vitamin A supplementation early in life on later asthma risk.

Methods: In 2006-2008, we revisited participants from two cohorts in rural Nepal enrolled in randomized trials of vitamin A supplementation. The first cohort received vitamin A or placebo for < 16 months during their pre-school years (1989-1991). The second cohort was born to mothers who received vitamin A, beta-carotene or placebo before, during and after pregnancy (1994-1997). At follow-up, we asked about asthma symptoms and performed spirometry.

Results: Of 6421 eligible to participate, 5430 (85%) responded to our respiratory survey. Wheezing prevalence during the past year was 4.8% in participants aged 9-13 years and 6.6% in participants aged 14-23 years. We found no differences between the vitamin A supplemented versus placebo groups from either trial in the prevalences of lifetime or current asthma and wheeze or in spirometric indices of obstruction ($p \geq 0.12$ for all comparisons).

Conclusions: Vitamin A supplementation early in life was not associated with a decreased risk of asthma in an area with chronic vitamin A deficiency.

INTRODUCTION

Asthma is a chronic lung disease that is associated with airway inflammation, airflow limitation, bronchial hyper-responsiveness and symptoms of episodic wheeze and cough. Its prevalence has increased rapidly over the past several decades in both developed and developing countries and it has emerged as one of the most prevalent non-communicable diseases worldwide. Asthma currently affects 300 million individuals worldwide, and is responsible for 180 thousand deaths and 15 million disability-adjusted life-years lost each year (1). The worldwide burden of disease caused by asthma is comparable to that of diabetes mellitus or cirrhosis (2, 3).

The increase in asthma prevalence is likely multi-factorial. Rapid urbanization and higher concentrations of outdoor air pollution in highly populated cities may have contributed to this rise (4, 5). A decrease in the incidence of infectious diseases may be associated with a shift in the cytokine balance and thus a greater risk of asthma, although this hypothesis remains unproven (6). Dietary intake has also been implicated in the etiology of asthma. In particular, a lower consumption of anti-oxidant vitamins may increase the vulnerability to oxidative stress, which may increase the risk or severity of asthma. Indeed, several observational studies have reported that lower levels of anti-oxidant vitamins C and E are associated with a greater risk of asthma (7 – 12). However, randomized controlled trials of supplementation with vitamins C and E on asthma risk have been inconclusive (13 – 15).

The causal relationship between vitamin A deficiency and asthma risk is less well characterized. Vitamin A is an anti-oxidant that plays a key role in lung development and regeneration of epithelial lung tissue (16, 17). We recently reported that offspring born to mothers who received vitamin A before, during and after pregnancy as part of a randomized trial had a greater lung function than did those whose mothers received placebo (18). Animal experiments have identified that vitamin A deficiency is associated with airway hyper-

responsiveness (19) and that supplementation with *all-trans* retinoic acid, an active metabolic intermediate of vitamin A, reverses airway hyper-responsiveness associated with vitamin A deficiency (20). While multiple observational studies (21 - 25) have found that vitamin A deficiency is associated with a higher risk of asthma, there are no randomized trials to support a causal relationship.

We undertook the present study to determine the effects of supplementation early in life with preformed vitamin A on subsequent asthma risk in an area with chronic vitamin A deficiency. To achieve this aim, we revisited participants from two cohorts of placebo-controlled vitamin A supplementation trials in rural Nepal. The first cohort received vitamin A or placebo during their pre-school years (26). The second cohort was born to mothers who received vitamin A, beta-carotene or placebo before, during and after pregnancy (27). Our hypothesis was that vitamin A deficiency early in life, which is known to affect pulmonary development adversely and promote airway hyper-responsiveness, may predispose to an increased risk of asthma during childhood through young adulthood.

METHODS

Study setting

We conducted our study in the Sarlahi District, located in the densely populated, low-lying southern plains in southern Nepal, known as the Terai. The Terai is an area of chronic undernutrition and vitamin A deficiency, where earlier surveys repeatedly observed childhood xerophthalmia to be a public health problem (28 – 30), attributable to early weaning from the breast and a traditional rice based diet low in animal, vegetable and fruit sources of vitamin A.

Original trials

The first trial (NNIPS-1) was a double-blind, placebo-controlled, cluster randomized trial between September 1989 and May 1991 to evaluate the effect of four-monthly, high potency vitamin A supplementation on pre-school child mortality (26). We mapped and addressed households in 29 contiguous village development committees (VDCs), each with 9 wards (our unit of randomization), and conducted a baseline enumeration at which time we enrolled children ≤ 60 months of age into the trial. On each subsequent 4-month visit, infants born during the previous interval were also enrolled. Ages of children were updated at each visit. On each occasion, children ≥ 12 months of age were given by trained field staff an oil-based supplement containing placebo or 200,000 IU of vitamin A (60,000 mcg retinol equivalents, RE), according to their ward assignment. Infants 1 - 11 months of age were offered a half-dose (placebo or 30,000 mcg RE) and newborns < 1 month of age a quarter-dose (placebo or 15,000 mcg RE). During a 16 month period we enrolled, dosed and followed a total of 28,630 children. Vitamin A was found to reduce the risk of childhood death by 30% after the fourth visit (26). Thereafter, all children aged 6 to 60 months received vitamin A supplementation throughout the last eight months of the trial while the youngest infants remained in the trial. As a result, the

supplementation intervals among children were variable. The majority of children received 4 doses over 16 months (>70%), whereas a smaller proportion received only one dose of vitamin A (<5%). We concurrently enrolled a subsample of 6,617 children into a more detailed substudy from 40 (15%) of the 261 wards that were randomly sampled within geographical quadrants of the study area (10 from each). Of these children, 6,462 (98%) were alive at the end of the trial.

The second trial (NNIPS-2) was a double-blind, placebo-controlled, cluster randomized trial of married women between April 1994 and September 1997 to evaluate the effect of weekly supplementation with vitamin A or beta-carotene on mortality from all causes related to pregnancy (27). We invited all eligible women from 30 VDCs to participate in this trial (total 270 wards, of which 261 were from NNIPS-1). The unit of randomization was the ward, and each ward within a VDC was block randomized to one of three treatments. We enrolled and continuously supplemented 44,646 women each week with an oral gelatin capsule containing 7,000 mcg RE of provitamin A, 42 mg of beta-carotene, or placebo. Supplementation with vitamin A or beta-carotene reduced pregnancy-related mortality by 44% versus placebo (27). Neither supplement had an effect on infant mortality (31). We concurrently enrolled pregnant women and their live born infants from a subsample of 3 contiguous VDCs (27 of 270 wards) into a more detailed clinical and biochemical assessment protocol. 2055 children were born alive to mothers in the subsample who completed the pregnancy-to-postpartum dosing protocols, of which 1894 (92%) were alive at the end of the trial. All children aged 6 to 60 months received biannual vitamin A supplementation through a national program.

Follow-up study

In 2006, we revisited the households of NNIPS-1 participants and offspring of NNIPS-2 mothers and invited them to participate in a follow-up study. We used a household list derived from the

original trials to generate an updated list of children who were eligible to participate in the follow-up study between October 2006 and March 2008. At follow-up, we conducted a survey in which we asked participants about respiratory symptoms (Supplementary Appendix). We selected the questions for our survey from recommended questionnaires (32, 33). We also asked participants to undergo spirometry using a portable spirometer (SpiroPro, Jaeger, Hoechberg, Germany). Each participant performed spirometry in the sitting position with a nose clip until three acceptable and reproducible maneuvers out of a maximum of eight were obtained, in accordance with standard guidelines (34). Technicians reviewed all of the flow-volume curves performed each day with a supervisor. Flow-volume curves were transmitted weekly to Baltimore for additional review. We performed direct supervision and in-person review of all flow-volume curves with each technician approximately every three months. This study was approved by the Institutional Review Boards of the Johns Hopkins University in Baltimore and the Institute of Medicine, Tribhuvan University in Kathmandu.

Biostatistical methods

We aimed to determine if there were differences in the prevalence of asthma or in measures of airflow limitation (35) at follow-up according to supplement assignment. We employed an intent-to-treat analysis and used generalized estimating equations for logistic regression with a compound symmetry correlation matrix (36) to account for clustering by ward. We calculated simple ward-level summaries for all outcomes and compared the ward-level means of FEV_1/FVC, peak expiratory flow (PEF), maximum midexpiratory flow (MMEF) and forced expiratory flow at 75% FVC (FEF75) by supplement assignment using standard techniques (37). Socioeconomic indicators, age or gender did not confound the effects of supplement assignment on outcome estimates (data not shown). Multivariable adjustment by height did not confound

the effect of supplement assignment on spirometric indices of obstruction (Supplementary Appendix). We used R (www.r-project.org) and SAS (Cary, NC, USA) for statistical analyses.

RESULTS

Characteristics of the NNIPS-1 study population

We summarized the status of participants from the NNIPS-1 original subsample aged 14 to 23 years at follow-up in Figure 1. Of 6462 children who were alive at the end of the trial, 1405 (21.7%) were no longer living in the study area and 275 (4.3%) had died. No information was available for 20 (0.4%) participants. 4523 were eligible to participate and 3879 (86% of those eligible) responded to our respiratory survey. 3345 attempted spirometry (74% of those eligible), and 3075 performed spirometry according to standard criteria. We did not find differences across study groups in the proportion of participants who died ($p=0.45$), who moved out of the study area ($p=0.58$), who did not complete the respiratory survey ($p=0.11$) or who refused to participate in the study ($p=0.69$). Participants who completed the respiratory survey were more likely to be of higher caste ($p=0.01$), to live in households that owned land ($p<0.001$), to own livestock ($p<0.001$), and to have a father who was a farmer ($p<0.001$) than the remaining 2738 participants from the original substudy. We did not find important differences in demographic or socioeconomic status between study groups at follow-up (Table 1).

Characteristics of the NNIPS-2 study population

We summarized the status of participants from the NNIPS-2 original subsample aged 9 to 13 years at follow-up in Figure 2. Of 1894 children who were alive at the end of the trial, 118 (6.2%) were no longer living in the study area and 109 (5.8%) had died. No information was available for 8 (0.4%) participants. 1659 children were eligible to participate and 1551 (93.5% of those eligible) responded to our respiratory survey. 1371 children attempted spirometry (83% of those eligible), and 1322 performed spirometry according to standard criteria. We did not find differences across study groups in the proportion of children who died before the close out of the

trial on 30 September 1997 (p=0.09), who died after 30 September 1997 (p=0.62), who moved out of the study area (p=0.78), who did not complete the respiratory survey (p=0.55) or who refused to participate in the study (p=0.40). Children who completed the respiratory survey were more likely to be of higher caste (p=0.04), to live in households that owned land (p<0.001), to own livestock (p<0.001), and to have a father who was a farmer (p<0.001) than the remaining 504 participants from the original substudy. We did not find important differences in demographic or socioeconomic status between study groups at follow-up (Table 2).

Prevalence of asthma

There was no difference in either the lifetime or current self-reported asthma in adolescent to adult participants who received vitamin A supplementation or placebo in their preschool years (Table 1) or in children born to mothers who received vitamin A supplementation, beta-carotene or placebo before, during and after pregnancy (Table 2) at follow-up. Among those who reported ever having asthma in either cohort, only 30% (26/88) reported being actually diagnosed by a physician or health worker, suggesting low access to medical personnel in the region. The low proportion of participants who reported having asthma in both cohorts *vis-à-vis* the higher prevalence of wheezing suggests that there is a large subset of individuals who have under-recognized, and possibly under-treated, asthma.

Prevalence of wheezing

The overall prevalence of wheezing in the past year was 6.6% (254/3852) in participants aged 14 to 23 years and 4.8% (73/1522) in participants aged 9 to 13 years at follow-up. There was no difference in the prevalence of either lifetime or current wheezing in adolescent and adult participants who received vitamin A supplementation or placebo in their preschool years (Table

1), or in children who were born to mothers who received vitamin A supplementation, beta-carotene or placebo before, during and after pregnancy (Table 2).. Of those who reported wheezing in the past year, 50.2% (164/327) also reported that they had at least one wheezing attack accompanied by shortness of breath and 45.0% (147/327) were awakening at night by wheezing. We did not find any differences between supplement assignment groups in either cohort (Tables 1 and 2) with respect to the number of attacks per year, wheezing accompanied by shortness of breath or wheezing-related night-time awakenings.

Prevalence of cough and phlegm

The prevalence of chronic productive and non-productive cough in both cohorts was 9 per 1000 participants (47/5427) and 13 per 1000 participants (70/5427), respectively. We did not find differences in cough or phlegm symptoms across study groups in either cohort (Tables 1 and 2). Stratified by sex and cohort, females aged 14 to 23 years had a similar prevalence of chronic productive cough as males (10 versus 12 per 1000 participants, respectively; p=0.64). Girls aged 9 to 13 years had a similar prevalence of chronic productive cough as boys of a similar age (4 versus 1 per 1000 participants, respectively; p=0.37).

Spirometric indices of obstruction

We display the distributions of FEV_1/FVC by sex and cohort in Figure 3. We did not find any differences in FEV_1/FVC, PEF, FEF75 or MMEF by supplement assignment in NNIPS-1 participants (Table 3). Despite a 46 ml increase in FEV_1 and FVC among offspring of women who received vitamin A versus placebo (18), there were no differences in FEV_1/FVC, PEF, FEF75 or MMEF across study groups (Table 4).

DISCUSSION

This study aimed to investigate the effect of vitamin A supplementation early in life on the subsequent risk of asthma in two cohorts of participants enrolled at different developmental periods in placebo-controlled vitamin A supplementation trials. In the first trial, a large dose of vitamin A or placebo was given to study participants every four months for sixteen months beginning when they were five years of age or younger, after which all children received prophylactic vitamin A until six years of age. In the second trial, vitamin A, beta-carotene, or placebo was given weekly to mothers of study participants before, during, and after pregnancy for approximately three years. After 1994, all pre-school aged children were subsequently exposed to a high-coverage semi-annual vitamin A supplementation as part of a national program (38). The results of our study indicate that vitamin A supplementation early in life did not affect the prevalence of asthma or measures of airways obstruction in participants aged 9 to 23 years.

There are several reasons why vitamin A deficiency, despite our findings, may be associated with a higher prevalence of asthma. Increased asthma might be expected in vitamin A deficient individuals because vitamin A is necessary for proper lung epithelial cell differentiation and lung development (39 - 41). Therefore, it would be reasonable to speculate that early lung development abnormalities may be implicated in the early origins of asthma. Animal studies have shown that vitamin A deficiency promotes airway hyper-responsiveness and alters the mechanical properties of the lung parenchyma. Vitamin A deficiency is also associated with a reduced abundance and function of muscarinic M_2 receptors, which may help explain the diminished ability to limit cholinergic-mediated bronchoconstriction (19). While supplementation with retinoic acid reverses the vitamin A-deficiency induced airway hyper-

responsiveness and the decrease in muscarinic M_2 receptors (20), it does not appear to reverse alterations in parenchymal mechanics and structure resulting from vitamin A deficiency (42).

Our findings, however, conflict with available epidemiological evidence from observational studies on the association between vitamin A and asthma (20 – 24). Thus, we need to consider reasons for why we may have not seen an effect of vitamin A supplementation on asthma. One possibility is that asthma may alter the metabolism of vitamin A. Factors implicated with asthma exacerbations, such as pulmonary infections or airways inflammation, have been shown to decrease serum retinol by increasing cellular demand or urinary excretion of retinol (23, 43 – 45). Thus, inflammation causing reduction in serum retinol may explain why observational studies have found an association between asthma and vitamin A (20 – 24) and would explain why vitamin A supplementation did not reduce the risk of asthma in either of our trials. Another possibility is that incomplete follow-up of the original trial participants because of loss to follow-up or death may have affected our findings. Our follow-up study included 59% of the original NNIPS-1 subsample and 75% of the original NNIPS-2 subsample. However, we did not find differences in characteristics participants according to supplement assignment at follow-up. A differential mortality between supplement allocation groups, as observed in NNIPS-1 but not NNIPS-2, could lead to a survivor bias where children who died might have been at greater risk of having asthma; however, the number of children who died in the NNIPS-1 trial was too small (11 to 16 deaths per 1000 child-years) (25) to result in an important survivor bias. A third possibility is that the duration of supplementation was relatively short: a maximum of 16 in NNIPS-1 trial participants, and during and shortly after pregnancy for the offspring of NNIPS-2 trial participants. Future studies may need to consider longer periods of vitamin A supplementation in the critical years of lung development (46, 47) to study the long-term effects of asthma risk. A fourth possibility is that implementation of a national vitamin A

supplementation program in pre-school aged children may have masked the effects of early childhood supplements provided during our trials. A fifth possibility is that early vitamin A supplementation affects lung growth later in life (18) by increasing both FEV_1 and FVC roughly equally, suggesting that lung size and airway caliber were symmetrically influenced by vitamin A supplementation. Finally, compliance with supplementation could have affected our results; however, this is unlikely because compliance in both trials was high. Specifically, compliance with vitamin A supplementation in the NNIPS-1 children was >90%, and over 75% of the NNIPS-2 pregnant women received at least half of their eligible doses of vitamin A or beta-carotene (i.e., more than half of a dietary allowance).

One limitation of this study is that we were not able to evaluate airway hyper-responsiveness or reversibility in this study. Airway hyper-responsiveness by means of a methacholine challenge would have provided additional data to support the relationship between vitamin A and asthma, but it would have been less feasible for us to perform given that our study was designed to visit participants at their homes. Furthermore, our study was not designed to have medically-trained personnel at all times in the field in case of any serious adverse events. Data regarding nutrition from birth to the time of follow-up and retinol levels at the time of follow-up were not available and would have helped us to better understand the relationship between asthma and vitamin A.

In summary, early vitamin A supplementation did not increase asthma risk. While vitamin A deficiency early in life affects pulmonary development adversely, it was not associated with an increased prevalence of asthma in an area with chronic vitamin A deficiency.

REFERENCES

1. Braman SS. The global burden of asthma. Chest. 2006; 130; 4–12.
2. Masoli M, Fabian D, Holt S, Beasley R. The global burden of asthma: executive summary of the GINA Dissemination Committee Report. Allergy 2004; 59: 469–78.
3. Bousquet J. The public health implications of asthma. Bull World Health Organ 2005; 83: 548–54.
4. Yemaneberhan H, Bekele Z, Venn A, Lewis S, Parry E, Britton J. Prevalence of wheeze and asthma and relation to atopy in urban and rural Ethiopia. Lancet 1997; 350: 85–90.
5. Committee of the environmental and occupational health assembly of the American Thoracic Society. Health effects of outdoor air pollution: part I of II. Am J Respir Crit Care Med 1996;153:3–50.
6. Busse W, Lemanske RF. Asthma. N Eng J Med 2001; 344: 350–62.
7. Schwartz J, Weiss ST. Dietary factors and their relation to respiratory symptoms. The Second National Health and Nutrition Examination Survey. Am J Epidemiol 1990; 132: 67–76.
8. Troisi RJ, Willett WC, Weiss ST, Trichopoulos D, Rosner B, Speizer FE. A prospective study of diet and adult-onset asthma. Am J Respir Crit Care Med 1995; 151: 1401–8.
9. Misso NL, Brooks-Wildhaber J, Ray S, Vally H, Thompson PJ. Plasma concentrations of dietary and nondietary antioxidants are low in severe asthma. Eur Respir J 2005; 26: 257–64.
10. Kelly FJ, Mudway I, Blomberg A, Frew A, Sandström T. Altered lung antioxidant status in patients with mild asthma. Lancet 1999; 354: 482–3.
11. Bodner C, Godden D, Brown K, Little J, Ross S, Seaton A. Antioxidant intake and adult-onset wheeze: a case-control study. Aberdeen WHEASE Study Group. Eur Respir J 1999; 13: 22–30.

12. Schünemann HJ, Grant BJ, Freudenheim JL, Muti P, Browne RW, Drake JA, Klocke RA, Trevisan M. The relation of serum levels of antioxidant vitamins C and E, retinol and carotenoids with pulmonary function in the general population. Am J Respir Crit Care Med 2001; 163: 1246–55.
13. Fogarty A, Lewis SA, Scrivener SL, Antoniak M, Pacey S, Pringle M, Britton J. Oral magnesium and vitamin C supplements in asthma: a parallel group randomized placebo-controlled trial. Clin Exp Allergy 2003; 33: 1355–9.
14. Pearson PJ, Lewis SA, Britton J, Fogarty A. Vitamin E supplements in asthma: a parallel group randomised placebo controlled trial. Thorax 2004;59: 652-6.
15. Romieu I, Sienra-Monge JJ, Ramírez-Aguilar M, Téllez-Rojo MM, Moreno-Macías H, Reyes-Ruiz NI, del Río-Navarro BE, Ruiz-Navarro MX, Hatch G, Slade R, Hernández-Avila M. Antioxidant supplementation and lung functions among children with asthma exposed to high levels of air pollutants. Am J Respir Crit Care Med 2002; 166:703-9.
16. Wilson JG, Roth CB, Warkany J. An analysis of the syndrome of malformations induced by maternal vitamin A deficiency. Effects of restoration of vitamin A at various times during gestation. Am J Anat 1953; 92: 189-217.
17. Chytil F. Retinoids in lung development. FASEB J 1996; 10: 986-92.
18. Checkley W, West KP Jr, Wise RA, Baldwin MR, Wu L, LeClerq SC, Christian P, Katz J, Tielsch JM, Khatry S, Sommer A. Effects of maternal vitamin A supplementation on lung function in preadolescent offspring: A follow-up study of a randomized, double-blinded, placebo-controlled trial cohort in rural Nepal. N Eng J Med 2010;362:1784-94.
19. McGowan SE, Smith J, Holmes AJ, Smith LA, Businga TR, Madsen MT, Kopp UC, Kline JN. Vitamin A deficiency promotes bronchial hyperreactivity in rats by altering muscarinic M_2 receptor function. Am J Physiol Lung Cell Mol Physiol 2002; 282: L1031-39.

20. McGowan SE, Holmes AJ, Smith J. Retinoic acid reverses the airway hyperresponsiveness but not the parenchymal defect that is associated with vitamin A deficiency. Am J Physiol Lung Cell Mol Physiol 2004; 286: L437-44.
21. Arora P, Kumar V, Batra S. Vitamin A status in children with asthma. Pediatr Allergy Immunol 2002; 13: 223–6.
22. Riccioni G, Bucciarelli T, Mancini B, Di Ilio C, Della Vecchia R, D'Orazio N. Plasma lycopene and antioxidant vitamins in asthma: the PLAVA study. J Asthma 2007; 44: 429-32.
23. Mizuno Y, Furusho T, Yoshida A, Nakamura H, Matsuura T, Eto Y. Serum vitamin A concentrations in asthmatic children in Japan. Pediatr Int 2006; 48: 261-4.
24. Morabia A, Menkes MJ, Comstock GW, Tockman MS. Serum retinol and airway obstruction. Am J Epidemiol 1990; 132: 77-82.
25. Allen S, Britton JR, Leonardi-Bee JA. Association between antioxidant vitamins and asthma outcome measures: systematic review and meta-analysis. Thorax 2009; 64: 610-9.
26. West KP Jr, Pokhrel RP, Katz J, LeClerq SC, Khatry SK, Shrestha SR, Pradhan EK, Tielsch JM, Pandey MR, Sommer A. Efficacy of vitamin A in reducing preschool child mortality in Nepal. Lancet 1991; 338: 67-71.
27. West KP Jr, Katz J, Khatry SK, LeClerq SC, Pradhan EK, Shrestha SR, Connor PB, Dali SM, Christian P, Pokhrel RP, Sommer A; NNIPS-2 Study Group. Double blind, cluster randomised trial of low dose supplementation with vitamin A or β-carotene on mortality related to pregnancy in Nepal. BMJ 1999; 318: 570-75.
28. Brilliant LB, Pokhrel RP, Grasset NC, Lepkowski JM, Kolstad A, Hawks W, Pararajasegaram R, Brilliant GE, Gilbert S, Shrestha SR. Epidemiology of blindness in Nepal. Bull World Health Organ 1985; 63: 375-86.

29. Upadhyay MP, Gurung BJ, Pillai KK, Nepal BP. Xerophthalmia among Nepalese children. Am J Epidemiol 1985; 121 :71-7.

30. Khatry SK, West KP Jr, Katz J, LeClerq SC, Pradhan EK, Wu LS, Thapa MD, Pokhrel RP. Epidemiology of xerophthalmia in Nepal. A pattern of household poverty, childhood illness, and mortality. The Sarlahi Study Group. Arch Ophthalmol 1995; 113: 425-9.

31. Katz J, West KP Jr, Khatry SK, Pradhan EK, LeClerq SC, Christian P, Wu LS, Adhikari RK, Shrestha SR, Sommer A. Maternal low-dose vitamin A or b-carotene supplementation has no effect on fetal loss and early infant mortality: a randomized cluster trial in Nepal. Am J Clin Nutr 2000; 71: 1570-76.

32. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, Mitchell EA, Pearce N, Sibbald B, Stewart AW, Strachan D, Weiland SK, Williams HC. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J 1995; 8: 483-91.

33. Ferris BG. Epidemiology standardization project (American Thoracic Society). Recommended respiratory disease questionnaires for use with adults and children in epidemiological research. Am Rev Respir Dis 1978; 118: 7–52.

34. American Thoracic Society. Standardization of Spirometry (1994 update). Am J Respir Crit Care Med 1995; 152: 1107-36.

35. Sears MR, Greene JM, Willan AR, Wiecek EM, Taylor DR, Flannery EM, Cowan JO, Herbison GP, Silva PA, Poulton R. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. N Engl J Med 2003; 349: 1414-22.

36. Liang KY, Zeger SL. Regression analysis for correlated data. Annu Rev Public Health 1993;14:43-68.

37. Hayes RJ, Moulton LH. Cluster randomized trials. Chapman & Hall/CRC Press, 2008. pp149-231.

38. Nepal Micronutrient Status Survey 1998. Kathmandu, Nepal: Ministry of Health, Child Health Division, HMG/N, NewERA, Micronutrient Initiative, UNICEF Nepal, and WHO; pp 54-66.

39. Wolbach SB, Howe PR. Tissue changes following deprivation of fat-soluble vitamin A. J Exp Med 1925; 42: 753-77.

40. Sharma HS, Mishra UK. Postnatal Distribution of Vitamin A in Liver, Lung, Heart and Brain of the Rat in Relation to Maternal Vitamin A Status. Biol Neonate 1986; 50: 345-350.

41. Zachman RD. Role of vitamin A in lung development. J Nutr 1995; 125:1634S–38S.

42. McGowan SE, Takle EJ, Holmes AJ. Vitamin A deficiency alters the pulmonary parenchymal elastic modulus and elastic fiber concentration in rats. Respir Res 2005:6:77.

43. Filteau SM, Morris S, Abbott RA, et al. Influence of morbidity on serum retinol of children in a community-based study in northern Ghana. Am J Clin Nutr 1993; 58:192–197.

44. Morabia A, Sorenson A, Kumanyika SK, Abbey H, Cohen BH, Chee E. Vitamin A, cigarette smoking, and airway obstruction. Am Rev Respir Dis 1989;140:1312-16.

45. Riccioni G, Barbara M, Bucciarelli T, di Ilio C, D'Orazio N. Antioxidant vitamin supplementation in asthma. Ann Clin Lab Sci 2007; 37: 96-101.

46. Galambos C, DeMello DE. Regulation of alveologenesis: clinical implications of impaired growth. Pathology 2008; 40: 124-40.

47. Burri PH. Structural aspects of postnatal lung development - alveolar formation and growth. Biol Neonate 2006;89:313-22.

Figure 1. Eligibility for census and enrollment of study participants 14 to 23 years of age from the original 6617 children of a well-defined subsample; NNIPS-1 follow-up study, Nepal, 2006 – 2008.

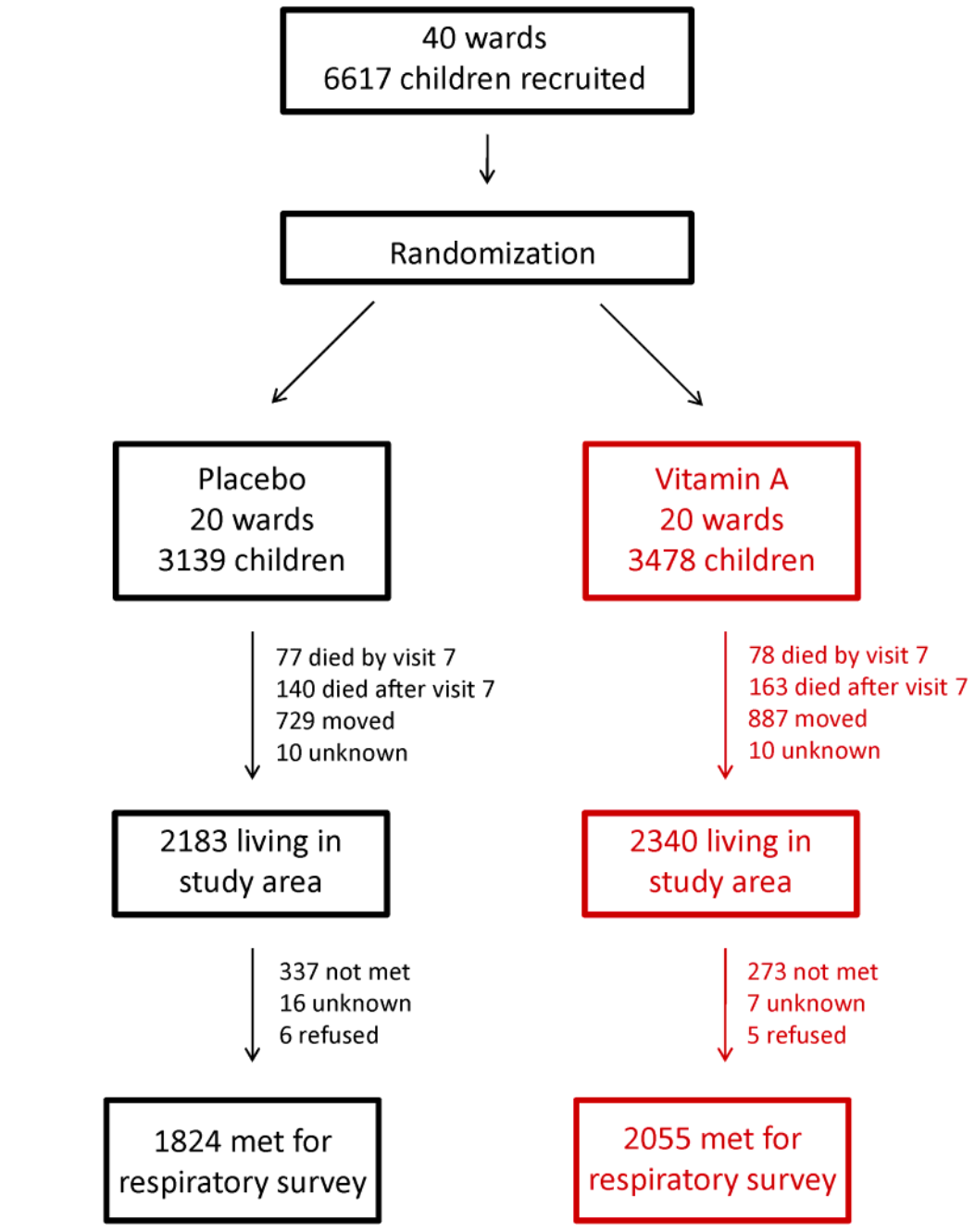

Figure 1.

Figure 2. Eligibility for census and enrollment of study participants 9 to 13 years of age from the original 2055 live births of a well-defined subsample; NNIPS-2 follow-up study, Nepal, 2006 – 2008.

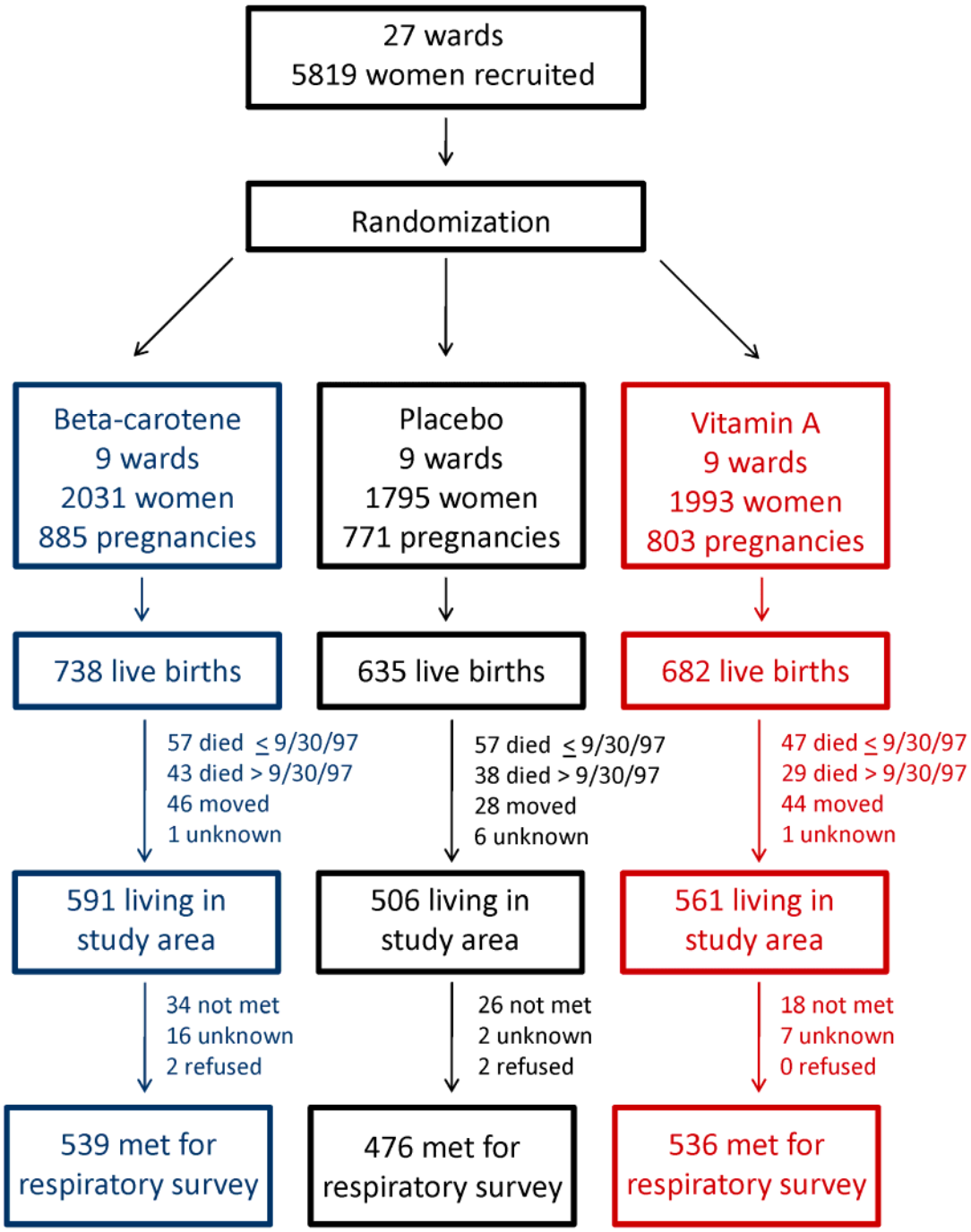

Figure 2.

Figure 3. Distribution of FEV_1/FVC (ratio of forced expiratory volume to forced vital capacity) in study participants 9 to 23 years of age; NNIPS follow-up study; Nepal, 2006 – 2008.

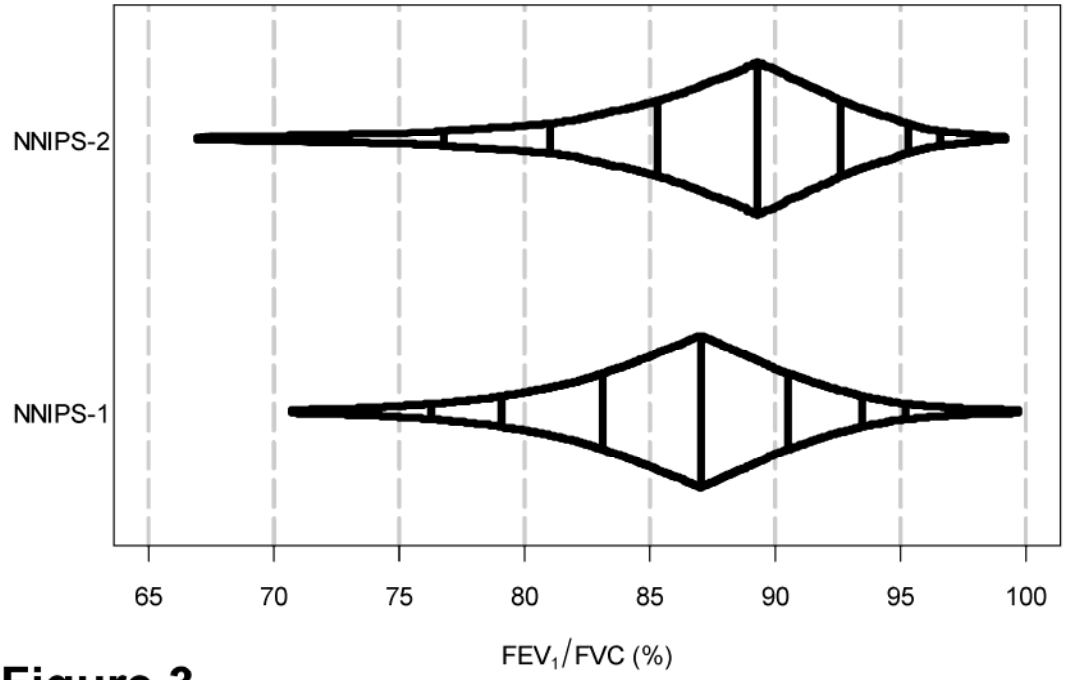

Figure 3.

TABLE 1. Sample size, demographics, socioeconomic indicators and prevalence of asthma, wheezing, and other respiratory symptoms in 3879 children who responded to the respiratory survey by supplement assignment group; NNIPS-1 follow-up study, Nepal, 2006 – 2008.

	Supplement assignment after birth		
	Placebo	**Vitamin A**	**p-value**
Sample size			
Number of wards	20	20	
Number of children	1824	2055	
Median (range) number of children per ward	78 (22–184)	83.5 (19–265)	
Demographics			
Mean age in years (SD)	18.7 (2.1)	18.7 (2.1)	0.73
Average of ward means (SD of means) of age in years	18.6 (0.3)	18.7 (0.3)	0.71
Sex (% males)	57	59	0.29
Average of ward proportions of males	58	59	0.40
Ward summaries of SES (%)			
Lower caste	81	84	0.66
Land ownership	81	83	0.74
Owns livestock	90	87	0.16
Owns radio	47	41	0.34
House without roof or with a thatch roof	40	36	0.74
House without walls or thatch-made walls	81	82	0.83
Maternal literacy	10	9	0.74
Father is farmer	70	66	0.34
Ward summaries of asthma prevalence (%)			
Lifetime asthma (self-report)	1.6	1.9	0.29
Physician diagnosis of asthma	0.8	0.6	0.75
Mean age in years (SD of means) at the onset of asthma	11.2 (6.0)	11.3 (6.0)	0.85
Current asthma (self-report)	0.6	0.6	0.98
Ward summaries of wheezing prevalence (%)			
Lifetime wheeze	11.3	12.2	0.24
Wheezing in the past year	6.7	6.5	0.85
Wheezing attacks in the past year			
1 – 3 (%)	4.0	3.7	0.46
4 – 12 (%)	2.1	2.2	0.80
12 or more (%)	0.9	0.9	0.69
Wheezing accompanied by shortness of breath in the past year	3.6	3.5	0.55
Disturbed sleep from wheezing in the past year			
Less than 1 night a week (%)	1.3	1.7	0.65
More than 1 night a week (%)	1.9	1.9	0.63
Ward summaries of cough and phlegm prevalence (%)			
Persistent cough (coughs more than normal)	7.3	7.0	0.83
Chronic cough (3 months of consecutive cough in a 2 year period)	2.6	2.2	0.70

	Average number of years (SD) with cough	2.8 (2.3)	4.2 (2.6)	0.08
	Persistent phlegm (produces phlegm more than normal)	12.4	10.3	0.29
	Chronic phlegm (3 months of consecutive phlegm production in a 2 year period)	3.7	3.3	0.50
	Chronic productive cough	1.2	1.0	0.60
	Dry cough at night in the past year	6.2	5.8	0.83
	Average number of years (SD) with phlegm	2.1 (1.2)	2.3 (1.3)	0.37
	Chest-illnesses after colds	23.1	24.9	0.49
	Mean number of phlegm episodes (SD) with chest illness	4.1 (5.7)	2.3 (1.2)	0.86

TABLE 2. Sample size, demographics, socioeconomic indicators and prevalence of asthma, wheezing, and other respiratory symptoms in 1551 children who responded to the respiratory survey by maternal supplement assignment group; NNIPS-2 follow-up study, Nepal, 2006 – 2008. "*" = could not estimate because of small sample size. "—" = No data available.

			Supplement assignment after birth			
			Beta-carotene	**Placebo**	**Vitamin A**	**p-value**
Sample size						
	Number of wards		9	9	9	
	Number of children		539	476	536	
	Median (range) number of children per ward		56 (29–105)	59 (23–87)	44 (16–134)	
Demographics						
	Mean age in years (SD)		11.0 (0.8)	11.0 (0.8)	11.0 (0.8)	
	Average (SD) of ward means of age		11.0 (0.2)	11.0 (0.2)	11.0 (0.2)	0.86
	Sex (% males)		53	47	51	
	Average of ward proportions of males		54	47	51	0.11
Ward summaries of SES (%)						
	Lower caste		86	91	84	0.37
	Land ownership		72	76	72	0.20
	Owns livestock		84	84	89	0.85
	Owns radio		31	29	31	0.65
	House without roof or with a thatch roof		24	25	24	0.99
	House without walls or thatch-made walls		91	96	89	0.07
	Maternal literacy		20	10	17	0.01
	Father is farmer only		51	55	46	0.12
Ward summaries of asthma prevalence (%)						
	Lifetime asthma (self-report)		0.5	0.5	1.1	0.12
	Physician diagnosis of asthma		0	0.2	0.1	0.59
	Mean age in years (SD) at the onset of asthma		2.5	—	3.8	*
	Current asthma (self-report)		0.2	0	0.7	*
Ward summaries of wheezing prevalence (%)						
	Lifetime wheeze		14.7	19.3	16.7	0.48
	Wheezing in the past year		5.5	3.7	5.3	0.20
	Wheezing attacks in the past year					
		1 – 3	3.7	2.0	2.7	0.09
		4 – 12	1.4	1.0	0.9	0.85
		12 or more	0.6	0.8	1.9	0.68
	Wheezing accompanied by shortness of breath in the past year		2.7	1.5	2.4	0.29
	Disturbed sleep from wheezing in the past year					
		Less than 1 night a week (%)	2.2	0.9	0.5	0.43
		More than 1 night a week (%)	2.1	1.5	1.6	0.52
Ward summaries of cough and phlegm prevalence (%)						
	Persistent cough (coughs more than normal)		4.8	3.6	5.5	0.23
	Chronic cough (3 months of consecutive cough		1.1	1.0	1.4	0.93

	in a 2 year period)				
	Average number of years (SD) with cough	1.5	2.7	0.7	0.14
	Persistent phlegm (produces phlegm more than normal)	5.9	3.9	7.8	0.14
	Chronic phlegm (3 months of consecutive phlegm production in a 2 year period)	2.1	0.3	1.7	0.03
	Chronic productive cough	0.5	0.1	0	0.76
	Dry cough at night in the past year	3.0	3.3	4.3	0.60
	Average number of years (SD) with phlegm	1.6 (1.0)	1.4 (0.7)	1.8 (1.9)	0.26
	Chest-illnesses after colds	23.5	21.2	20.0	0.96
	Mean number of phlegm episodes (SD) with chest illness	1.7 (1.6)	0.3 (—)	0.6 (0.5)	0.02

TABLE 3. Spirometric indicators of obstructive airways disease in 3075 children by supplement assignment group; NNIPS-1 follow-up study, Nepal, 2006 – 2008.

		Supplement assignment after birth		
		Placebo	**Vitamin A**	**p-value**
Sample size for boys				
	Number of boys	804	934	
	Median (range) number of boys per ward	35.5 (12–76)	40.5 (9–112)	0.41
FEV_1/FVC for boys				
	Average (SD) of FEV_1/FVC (%)	85.9 (6.1)	86.1 (5.8)	0.58
	Ward means (SD of means) of FEV_1/FVC (%)	85.7 (1.4)	86.1 (1.0)	0.35
PEF for boys				
	Average (SD) of PEF (L/s)	7.81 (1.69)	7.85 (1.56)	0.66
	Ward means (SD of means) of PEF (L/s)	7.67 (0.67)	7.80 (0.33)	0.44
FEF75 for boys				
	Average (SD) of FEF75 (L/s)	1.77 (0.63)	1.80 (0.63)	0.43
	Ward means (SD of means) of FEF75 (L/s)	1.74 (0.19)	1.79 (0.13)	0.39
MMEF for boys				
	Average (SD) of MMEF (L/s)	3.66 (0.96)	3.69 (0.95)	0.59
	Ward means (SD of means) of MMEF (L/s)	3.60 (0.32)	3.67 (0.16)	0.41
Sample size for girls				
	Number of girls	629	708	
	Median (range) number of girls per ward	30 (4–67)	26.5 (6–97)	0.57
FEV_1/FVC for girls				
	Average (SD) of FEV_1/FVC (%)	87.4 (5.6)	87.2 (5.9)	0.59
	Ward means (SD of means) of FEV_1/FVC (%)	87.6 (1.3)	87.1 (1.4)	0.28
PEF for girls				
	Average (SD) of PEF (L/s)	5.53 (1.11)	5.61 (1.13)	0.18
	Ward means (SD of means) of PEF (L/s)	5.46 (0.33)	5.48 (0.45)	0.87
FEF75 for girls				
	Average (SD) of FEF75 (L/s)	1.39 (0.49)	1.39 (0.51)	0.94
	Ward means (SD of means) of FEF75 (L/s)	1.37 (0.16)	1.35 (0.17)	0.74
MMEF for girls				
	Average (SD) of MMEF (L/s)	2.93 (0.73)	2.95 (0.75)	0.65
	Ward means (SD of means) of MMEF (L/s)	2.88 (0.26)	2.89 (0.28)	0.98

TABLE 4. Spirometric indicators of obstructive airways disease in 1322 children by maternal supplement assignment group; NNIPS-2 follow-up study, Nepal, 2006 – 2008.

	Supplement assignment after birth			
	Beta-carotene	**Placebo**	**Vitamin A**	**p-value**
Sample size for boys				
Number of boys	254	202	226	
Median (range) number of boys per ward	25 (14–44)	22 (9–45)	19 (5–70)	0.71
FEV_1/FVC for boys				
Average (SD) of FEV_1/FVC	87.5 (7.1)	87.6 (6.1)	87.8 (6.0)	0.88
Ward means (SD of means) of FEV_1/FVC	87.6 (1.3)	87.5 (1.8)	88.1 (1.2)	0.66
PEF for boys				
Average (SD) of PEF (L/s)	3.66 (0.91)	3.78 (0.82)	3.79 (0.75)	0.17
Ward means (SD of means) of PEF (L/s)	3.69 (0.19)	3.91 (0.33)	3.86 (0.23)	0.18
FEF75 for boys				
Average (SD) of FEF75 (L/s)	0.86 (0.34)	0.83 (0.30)	0.89 (0.33)	0.15
Ward means (SD of means) of FEF75 (L/s)	0.87 (0.07)	0.83 (0.06)	0.89 (0.06)	0.17
MMEF for boys				
Average (SD) of MMEF (L/s)	1.93 (0.59)	1.92 (0.52)	1.98 (0.53)	0.44
Ward means (SD of means) of MMEF (L/s)	1.94 (0.13)	1.95 (0.13)	2.01 (0.14)	0.52
Sample size for girls				
Number of girls	209	217	214	
Median (range) number of girls per ward	22 (12–47)	26 (7–45)	19 (6–55)	0.99
FEV_1/FVC for girls				
Average (SD) of FEV_1/FVC (%)	89.1 (6.6)	88.9 (6.8)	89.4 (6.1)	0.75
Ward means (SD of means) of FEV_1/FVC (%)	89.0 (2.2)	88.7 (1.6)	89.4 (2.2)	0.75
PEF for girls				
Average (SD) of PEF (L/s)	3.45 (0.79)	3.50 (0.88)	3.49 (0.74)	0.80
Ward means (SD of means) of PEF (L/s)	3.44 (0.30)	3.61 (0.47)	3.47 (0.19)	0.55
FEF75 for girls				
Average (SD) of FEF75 (L/s)	0.85 (0.34)	0.81 (0.32)	0.85 (0.32)	0.47
Ward means (SD of means) of FEF75 (L/s)	0.84 (0.12)	0.84 (0.13)	0.86 (0.12)	0.90
MMEF for girls				
Average (SD) of MMEF (L/s)	1.90 (0.55)	1.86 (0.57)	1.93 (0.51)	0.45
Ward means (SD of means) of MMEF (L/s)	1.89 (0.21)	1.90 (0.23)	1.93 (0.16)	0.92