



Vitamin A Status, Reproductive Health and Survival: The Nepal Nutrition Intervention Programme

INTRODUCTION

There can be little doubt that the available scientific evidence supports, very strongly, the association of oral vitamin A supplements administered to children who are deficient in vitamin A and a significant reduction in child mortality¹. Less attention, however, has so far been afforded to the consequences of vitamin A deficiency on maternal health and survival. Maternal vitamin A deficiency is increasingly being recognised as a major public health problem in many developing countries, but its consequences have so far been assumed to be mainly related to infant health status, morbidity, and mortality. One symptom of vitamin A deficiency, night blindness, has been estimated to affect 1 to 2 million currently pregnant women in Southern Asia alone and is reported to be associated with an increased health risk to women².

In the southern plains of Nepal, an area of the country in which the administration of vitamin A supplements has been shown to be associated with a reduction in child mortality of 30 percent³, 10 to 20% of women develop nightblindness during pregnancy; furthermore, maternal and infant mortality are also 65 and 10 times higher in Nepal, respectively, than the levels observed in most developed countries. In this setting, a research team from Johns Hopkins University in the United States and the National Society for Eye Health and Blindness Prevention in Nepal have completed a large randomised field trial, called NNIPS-2 (Nepal Nutrition Intervention Project, Sarlahi-2), to examine whether maternal, fetal, or infant mortality can be lowered by providing women of childbearing age a weekly supplement, approximating the current Recommended Dietary Allowances (RDAs), of vitamin A before, during, and after pregnancy. A summary of selected findings from this survey, which were presented at the XVIII IVACG Congress⁴ in Cairo, Egypt, in September last year, form the focus of this Update.

Survey Design

The major goal of this randomised, double-blind community based trial was to evaluate the impact of weekly supplementation with vitamin A (VA), beta-carotene (BC) or placebo (PL) (Table 1) on mortality of women of reproductive age, fetal loss, infant mortality, intrauterine and postnatal growth, specific micronutrient (e.g. vitamin A and iron) and general nutrition.

Table 1. Survey design

Beta-carotene group	Vitamin A group	Placebo group
7,000 RE/week	7,000* RE/week	0 RE/week
90 Wards	90 Wards	90 Wards
n=12,500	n=12,500	n=12,500
	* as retinyl palmitate	

Subjects were married women aged 13-45 years living with their husbands. Newly married women were concurrently enrolled as the study progressed. Four hundred and thirty women were employed as ward distributors to provide the weekly coded supplements, to record the

receipt of the supplement, the date of menstruation, pregnancy status, and when pregnant, to ask about nightblindness. Following randomisation, women who declared a pregnancy completed an interview schedule at home, once during the second and once during the third trimester of pregnancy, and at 3 and 6 months postpartum. Seven-day histories about morbidity, dietary intake and other lifestyle factors including smoking, alcohol consumption, mid-upper arm circumference (MUAC) and verbal autopsies were carried out for maternal and infant deaths.

A 10% sub-sample of pregnant women residing in 3 Village Development Communities (VDCs) from 27 wards were examined in a mid-pregnancy clinic visit and at 3-4 months postpartum with their infants to assess health and micronutrient/nutritional status. The sub-sample was examined at home within 3-10 days postpartum to note birth defects and at 10-20 days and 6 months postpartum by an anthropometry team to assess infant growth by weight, length, MUAC and skinfolds. Infant and maternal deaths were investigated by verbal autopsy.

At clinic visits, a physical examination was carried out, anthropometric measurements of weight, height, skinfold and MUAC were done and blood samples were taken for the determination of vitamin A, iron and malaria status. Stool samples were also taken to determine the presence or absence of helminth infection. Breastmilk retinol and carotenoids were measured. The survey also included a dietary assessment, conjunctival impression cytology and iodine status assessment by urinary iodine. All data were recorded and entered onto databases. In all, 44 272 women were recruited (36 828 at baseline, 7 444 prospectively, of those 1,000 migrated) and 20 000 pregnancies were reported.

Treatments were given as capsules. The bottles were inspected for consumption of capsules to measure compliance. It was calculated that 80% of women received at least half of all their weekly doses; therefore, all subjects received at least half the RDAs.

Impact of Supplementation on Fetal, Infant and Maternal Mortality

Although the results presented at the Congress were preliminary, the trialists did not expect the final conclusions to be significantly different. All the women allocated to the different study groups were comparable and came from comparable households. An evaluation of the 7-day food frequency questionnaires at mid-pregnancy showed that 50% of the women ate meat/fish/eggs, 60% ate dairy products, 15% ate yellow vegetables, 30% smoked 'bidi', 10% drank local spirit, and 15% carried firewood.

The prevalence of low serum retinol (<0.7 umol/L) at mid-pregnancy was 7 times lower in the VA group and about 1.5 times lower in the BC group as compared with the PL group. By three months postpartum the risk of low serum retinol was 5.5 times lower in the VA group and twice as low in the BC group as compared with the PL group.

Although both vitamin A (43%) and beta-carotene (55%) supplements appeared to reduce mortality during pregnancy, the small number of deaths in all 3 groups probably prevented the result being significant. However, the combined impact on all causes of pregnancy-related mortality up to 12 weeks postpartum was 713, 443 and 401 deaths per 100,000 in the PL, VA and BC groups respectively. This data yielded relative risks (RR) of 0.62 [95%, confidence intervals (CI): 0.4, 0.96] and 0.50 (95%, CI: 0.31, 0.80) for the VA and BC groups respectively. Pregnancy-related mortality was, therefore, decreased by 38% with vitamin A and by 50% with beta-carotene supplementation. Combining the intervention arms of the trial, i.e. VA + BC, reduced pregnancy-related mortality by 44% after correction for ward randomisation. In terms of weekly morbidity, relatively small changes were observed across treatment groups during and after pregnancy. However, vitamin A (but not beta-carotene) supplementation reduced the prevalence of malarial infection by 40% (from 23% to 13%), a statistically significant impact. The malaria transmitted in this region was exclusively *Plasmodium vivax*. There was no impact of supplementation on stillbirths or miscarriages or on infant mortality below 6 months of age.

In summary, in these Nepalese women, who are chronically vitamin A deficient, a weekly beta-carotene or vitamin A supplement of 7,000 RE/week would appear to reduce pregnancy-related mortality by 30-50%.

Impact of Supplementation on the Incidence of Nightblindness during Pregnancy and Lactation

The incidence of nightblindness was determined by history and was assessed in 9 932 women who participated in the trial. The history was obtained using a local term 'ratauni' at the time of the distribution of the supplements in 19 of the participating 30 wards. In all, 868 out of approximately 10,000 women reported nightblindness at least once. Overall, women reporting nightblindness had lower mean serum retinol and haemoglobin concentration as well as a poorer nutritional status (Table II).

Table II. Nutritional parameters in women reporting nightblindness

Parameter	Case mean	Control mean	Mean difference
Height (cm)	149.	151.0	-1.30
BMI	20.	20.9	-0.79*
MUAC (cm)	21.	22.7	-0.88*
Hb (g/dL)	8.9	9.6	-0.67*
Retinol (umol/L)	0.72	1.03	-0.30*
Weight (kg)	45.2	47.7	-2.57*
*p< 0.001			

The incidence of nightblindness among pregnant women was 6.7%, 8.9% and 10.7% in the VA, BC and PL groups, respectively, yielding RR of 0.62 (95%, CI: 0.53, 0.73) in the VA and 0.84 (95%, CI: 0.72, 0.90) in the BC group. The impact of vitamin A and beta-carotene supplementation on nightblindness at three months postpartum showed a RR of 0.38 (95%, CI: 0.28, 0.52) in the VA and 0.78 (95%, CI: 0.61, 1.0) in the BC group; by 4-6 months postpartum the RR was 0.41 (95%, CI: 0.29, 0.58) and 0.66 (95%, CI: 0.54, 0.82) in the respective groups. Therefore, the incidence of nightblindness during the first six months postpartum was reduced by approximately 60% and 30% in the VA and BC groups respectively. There was a tendency for nightblindness to be reported earlier in pregnancy in the PL group than in the other two groups, and supplementation was more protective in the dry and post-harvest seasons when food availability was lower. Also of importance, was the finding that the protective effect of vitamin A or beta-carotene supplements increased with compliance. If compliance (number of doses from last menstrual cycle through pregnancy) was less than 65% of prescribed treatment, there was no protection against nightblindness (RR>0.96). If compliance, however, was greater than 65%, the RR of nightblindness decreased to 0.49 (96-100% compliance). Women with poor levels of compliance were also likely to have been poor participants, with less reliable histories, i.e. under-reporting. Furthermore, among women with nightblindness, death rate was 25.6/1,000 compared with 3.4/1,000 in those without nightblindness; thus, nightblindness was associated with a 7.5 times higher risk of dying (95%, CI: 3.06,18.27).

In summary, vitamin A supplements in this study is reported to decrease the incidence of nightblindness by 40%; beta-carotene is also reported to be a less effective supplement but, nevertheless, reduces nightblindness by 16-25%. Importantly, high compliance may result in a 60-65% reduction in nightblindness. Equally importantly, maternal nightblindness is reported to be associated with a 7.5 times higher risk of dying.

Impact of Supplementation on Iron Deficiency Anaemia (IDA) in Pregnant Women, Postpartum Mothers and Infants

An investigation as to whether vitamin A or beta-carotene supplementation had an effect on iron status and anaemia in women and infants as well as the influence of hookworm and

malaria on these parameters was also included in the trial. Clinical studies were done on a sub-sample of 10% of all women who completed clinical assessments. Results were presented from 978 pregnant women, 766 postpartum mothers and 728 infants. Women were invited to the clinic for a pregnancy assessment and a 3-month postpartum assessment, which included their infants. Haemoglobin (Hb), erythrocyte protoporphyrin (EPP) and serum ferritin were measured. Parasitic infestation was assessed only in pregnancy. Malaria parasitaemia was determined using a thin blood film, and hookworm infection was determined by faecal egg counts in 622 (64%) of the pregnant women (those who complied) using the Kato-Katz method.

The characteristics of treatment groups were similar in height and weight but supplementary iron use was lower in the placebo group (0.4%) compared to 2.1 % and 2.4% in the VA and BC groups respectively. Anaemia was present in 71% of pregnant women, 78% of postpartum mothers and 57% of infants. In women who received vitamin A supplements, anaemia was reduced by 9% during pregnancy and postpartum; haemoglobin concentration of their infants was only slightly increased (0.2g/dL) relative to the PL group (Tables III-V). Serum ferritin in infants was uniformly high and was not affected by either vitamin A or beta-carotene supplementation.

Table III. Indicators of iron status during pregnancy

Indicator	Placebo	Vitamin A	Beta-carotene
Mean Hb g/dL (SD)	9.9 (1.1)	10.2 (1.5)	10.1 (1.6)
Hb % <11 g/dL	76.0	68.4	68.7
% <9 g/dL Hb	20.7	19.8	20.2
EPP umol/mol (SD)	97 (65)	94 (61)	101 (65)
Ferritin ug/L (SD)	11.3 (11.6)	13.3 (12.8)	13.1 (15.9)
% IDA*	62.7	52.3	58.0
IDA (iron deficiency anaemia) = Hb <11, EPP >90, Ferritin <12			

Table IV. Indicators of iron status postpartum

Indicator	Placebo	Vitamin A	Beta-carotene
Mean Hb g/dL	10.6	10.7	10.6
% <11 g/dL Hb	82.9	75.2	77.8
% <9 g/dL Hb	13.3	13.4	16.3
EPP umol/mol (SD)	12.6 (11)	17.4 (17.2)	14.8 (14.9)
Ferritin ug/L (SD)	11.2 (7.8)	11.7 (8.4)	11.4 (7.2)
% IDA	68.2	63.4	55.7
IDA (iron deficiency anaemia) = Hb <11, EPP >90, Ferritin <12			

Table V. The effect of maternal supplementation on the Hb values of infants

indicator	Placebo	Vitamin A	Beta-carotene
Hb mean (SD)	10.7 (1.1)	10.9 (1.3)	10.7 (1.2)
% <12 g/dL Hb	89.9	80.1	87.6
% <11 g/dL Hb	61.4	55.2	55.9

Plasmodium vivax parasitaemia was found in 23% of women who received placebo or beta-carotene but in only 14 % of women who received vitamin A ($p < 0.08$). Thus, the vitamin A impact on anaemia might in part have been mediated by decreased malarial infection. The RR for anaemia in pregnant women in the VA group with or without adjustment for malarial outcome was 0.94 (95%, CI: 0.84, 1.05). The RR for IDA in pregnant women in the VA group was 0.85 (95%, CI: 0.73, 1.00) without adjustment and 0.86 (95%, CI: 0.74, 1.01) with adjustment for malaria

Hookworm (*Ancylostoma duodenale*) infection was equally prevalent in all three groups (77%), was strongly associated with IDA and modified the effect of vitamin A on iron status. In women with no hookworm, the prevalence of women with Hb <10 g/dL was 50% and 40% lower, elevated EPP (>90 $\mu\text{mol/mol}$) was 44% and 54% lower, and IDA was 46% and 27% lower in the VA and BC groups respectively compared with the PL group. The impact of vitamin A supplementation in hookworm-infected women was small, and was apparently overcome by the effect of blood loss.

In summary, vitamin A and beta-carotene supplementation lowered the risk of moderate (not severe) maternal anemia (hemoglobin < 10 g/dL) by 45% in the one fourth of women who were free of hookworm, an impact of biologic and statistical significance. However, women with hookworm infestation (the vast majority of women) showed no discernible hemoglobin response to either supplement. In multivariate analyses, the impact on hemoglobin (which was observed in both supplemented groups) did not appear to be explained by the decrease in malarial infection found in the vitamin A group. Among infants of supplemented mothers, a slight reduction in the percentage of those with Hb < 11 g/dL was observed.

Impact of Supplementation on the Incidence of Birth Defects among Infants

The reported, and extensively debated, predisposition to teratogenicity by daily vitamin A supplements in excess of 10 000 IU during pregnancy has been proposed to be possibly related to resultant high circulating levels of the potentially teratogenic *all-trans* retinoic acid (RA), *13-cis* RA and their metabolites *4-oxo-all-trans* RA and *4-oxo-13-cis* RA. This study used a supplementary dose of 23,300 IU/week and its aim was to determine the effects of long-term, low, weekly doses of vitamin A or beta-carotene (7,000 RE/week) on 276 pregnant (2nd trimester) and 103 lactating women (3-4 months postpartum). Blood was drawn and the serum transported under liquid nitrogen to Johns Hopkins University, where retinol, beta-carotene, alpha-carotene and other carotenoids were measured using a reverse phase isocratic HPLC. Aliquots of plasma were also sent to Roche laboratories, Basle, where RA metabolites were determined with an automated HPLC method employing gradient elution and using a column-switching technique. The trial also monitored the efficacy of supplementation in preventing birth defects.

Concentrations of serum *all-trans* RA were within the normal range (normal range 1.2-1.81 ng/ml) while serum *13-cis* RA (normal range 1.4-1.6 ng/ml) and *4-oxo-3-cis* RA (normal range 2.72-3.68 ng/ml) tended to be lower than the normal range during pregnancy and postpartum. Time-dependent differences in RA by the number of days post-supplementation showed no peak in *all-trans* RA concentration, while *13-cis* RA concentration peaked the first day following dosing, and then decreased over the next 35 days in the VA group. In the latter group, *13cis-oxo-RA* peaked at day 1, then within 2 days the levels decreased. Weekly dosing with vitamin A or beta-carotene significantly increased serum retinol concentration (Table VI), but *all-trans* RA did not increase. Although *13-cis* RA and *4-oxo-13-cis* RA

concentrations did increase in the VA group as compared with the PL or BC groups, these concentrations were still lower than those found in well-nourished individuals.

Table VI. Serum retinol (ug/dL) in supplemented pregnant women or postpartum

Group	Placebo	Vitamin A	Beta-carotene
Pregnancy	29.1	37.3	32.7
Postpartum	27.3	42.8	34.5

In terms of birth defect prevention, the study reported significantly lower risks of ocular birth defects in the VA group (RR=0.17). Vitamin A was also strongly protective against visual impairment (RR=0.12). Severe cranial and ocular defects were reduced in the vitamin A group, but the presence of integumental birth defects such as naevi, haemangiomas and cysts were not statistically different.

In summary, the data from this trial suggest that routine supplementation of women during pregnancy and lactation with 7,000 RE as a single, oral, weekly bolus of preformed vitamin A, or a provitamin A carotenoid, in chronically malnourished populations, appears to be safe and to have little measurable effect on circulating levels of potentially teratogenic retinoids.

OTHER FINDINGS

Despite improvements in maternal health and survival, neither vitamin A nor beta-carotene supplementation reduced fetal or infant mortality through to 6 months of age. Supplementation had also no effect on the neonatal weight of infants, although a small improvement in ponderal growth and reduction in lower respiratory tract infection were observed during the first 3 months of life.

CONCLUSION

This large, well-conducted, randomised, double blind, long-term community based trial is likely to be another landmark in the science of nutrition. It confirms the importance of vitamin A not only in its well-known function in vision but also in its “newly” documented role in reducing child mortality. The trial extends the importance of the vitamin to maternal health and well being. One, of course, must await the full publication of the data and the necessary confirmation of these preliminarily reported findings. Nevertheless, the findings presented at the Congress, although insufficient for general public health policy formulation, are sufficiently robust to warrant, in terms of safety and benefit, serious consideration regarding the possible supplementation of high-risk, chronically malnourished women.

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