Effectiveness of an early supplementation scheme of high-dose vitamin A versus standard WHO protocol in Gambian mothers and infants: a randomised controlled trial

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Summary

Background **Most developing countries have adopted a standard WHO dosing schedule for vitamin A supplementation. However, in 2002 the International Vitamin A Consultative Group (IVACG) Annecy Accord recommended a new high-dose regimen for mothers and infants. Our aim was to test whether the new high-dose regimen of vitamin A supplementation would increase maternal and infant plasma vitamin A, reduce infant *Helicobacter pylori* infection and nasopharyngeal pneumococcal carriage, and improve infant gut epithelial integrity.**

Methods **In an area of moderate vitamin A deficiency in rural Gambia, 220 mother–infant pairs were enrolled in a randomised double-blind trial between September, 2001, and October, 2004, that compared the IVACG high dose with the WHO dose. The primary endpoints were levels of maternal and infant plasma vitamin A, *H pylori* infection, pneumococcal carriage, and gut epithelial integrity. The trial is registered as ISRCTN 98554309.**

Findings **197 infants completed follow-up to 12 months (99 high dose and 98 WHO dose). There were no adverse events at dosing. No differences were found in the primary outcomes for high-dose versus WHO schedule: maternal vitamin A concentration at 2 months +0·02 µmol/L (95% CI −0·10 to 0·15); infant vitamin A at 5 months +0·01 µmol/L (−0·06 to 0·08); *H pylori* infection at 12 months −0·3% (−14·7 to 14·2); maternal pneumococcal carriage at 12 months −2·0% (−13·7 to 9·7); infant pneumococcal carriage at 12 months −4·1% (−15·8 to 7·6); infant gut mucosal damage at 12 months 5·2% (−8·7 to 19·2). There were more clinic attendances by the high-dose group in the first 6 months of life (p=0·018).**

Interpretation **Our results do not lend support to the proposal to increase the existing WHO standard dosing schedule for vitamin A in areas of moderate vitamin A deficiency. Caution is urged for future studies because trials have shown possible adverse effects of higher doses of vitamin A, and potential negative interactions with the expanded programme on immunisation (EPI) vaccines.**

Introduction

Meta-analyses show that vitamin A supplementation of preschool children in vitamin A deficient populations reduces all-cause mortality by 30%.[1,2] Supplementation with a standard WHO protocol (200 000 IU to mothers early postpartum, 100 000 IU to infants at 9 months, and 200 000 IU at 4–6 month intervals thereafter) has been adopted as national policy in most developing countries.[3,4]

Initially, supplements were not given to young infants because of concerns about possible toxicity.[5] However, WHO sponsored a multi-centre trial of 200 000 IU of vitamin A to mothers early postpartum, and 25 000 IU to infants at their three initial expanded programme on immunisation (EPI) visits at 6, 10, and 14 weeks.[6] The trial did not detect toxicity and did not show any increase in plasma vitamin A, growth, or morbidity. This disappointing result prompted the International Vitamin A Consultative Group (IVACG) to recommend a higher dosing schedule of two 200 000 IU doses to mothers early postpartum, and 50 000 IU to infants at their EPI visits.[7] Doses were calculated to build up body stores in babies who were born to vitamin A deficient mothers.[8] The proposed high-dose regimen has not been tested formally, and a recent trial has suggested that lower doses than WHO standard regimen might preferentially reduce mortality in older children (6 months to 5 years).[9] This result highlights the need to understand better the mechanisms by which vitamin A exerts its effect on mortality.

We used a randomised double-blind controlled trial to compare the efficacy of the IVACG early high-dose protocol with that of the WHO protocol by the assessment of adverse events at dosing, maternal and infant vitamin A concentrations, mucosal integrity, growth and morbidity patterns, and measurements of infant immunity.

Methods

Study design

The study was done in Keneba and five surrounding villages in the West Kiang District of The Gambia. Descriptions of this area, with special reference to vitamin A status, have been published elsewhere.[10,11] All pregnant mothers were identified by the local mobile midwifery team and provisionally enrolled at 30 weeks'

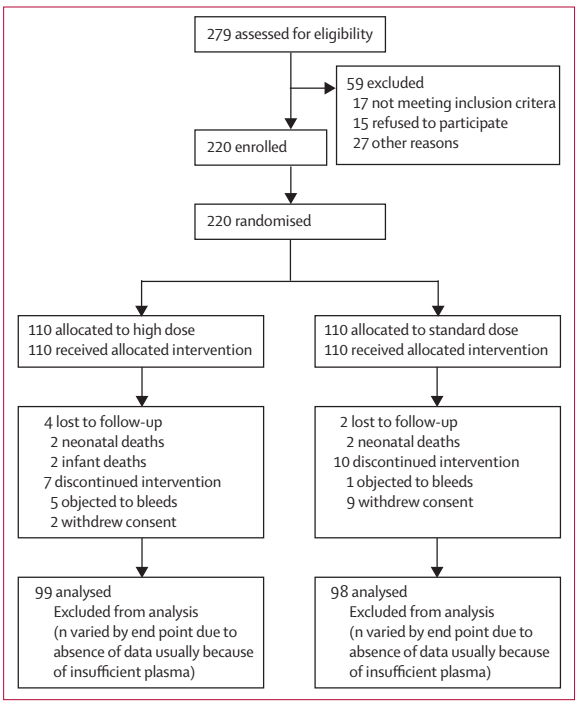

Figure 1: **CONSORT flowchart**

gestation. Recruitment of patients was completed at delivery after appropriate consent procedures, if a cord blood sample was successfully obtained and in the absence of these exclusion criteria: weight at birth less than 2200 g, premature delivery (<37 completed weeks), and congenital birth defects or severe peripartum difficulties.

Figure 1 shows the trial profile. Between September, 2001, and October, 2004, 220 mother–infant pairs were randomly assigned to either the IVACG early high dose[7] or the standard Gambian Government supplement (equivalent to the standard WHO protocol).[3] Table 1 shows details of high-dose and WHO-dose interventions. Vitamin A in vegetable oil and vegetable oil placebo were prepared by Hoffmann La Roche (Basel, Switzerland). An independent senior scientist packed and labelled the supplements, and did a block randomisation procedure (16 per block) to allow for possible effects of season of birth. Supplements or placebo were directly administered at home by field staff, apart from supplements at 2, 9, and 12 months, which were given at the clinic during follow-up. All members of the trial team were unaware of allocation until the data had been cleaned and locked.

The study was approved by the joint MRC Gambia and Gambian Government, and LSHTM Ethics Committees. Field procedures were approved by the National Nutrition Agency (NaNA) and Divisional Health Team for Lower River Division. For the duration of the study, the trial team acted in lieu of government personnel in the distribution of vitamin A supplements. Adverse events were monitored continuously by the trial physician. There were no formal stopping rules.

	Dose (IU)	
	High dose	Standard WHO dose
Mothers		
At delivery	200 000	200 000
Within a week	200 000	Placebo
Infants		
2 months	50 000	Placebo
3 months	50 000	Placebo
4 months	50 000	Placebo
9 months	100 000	100 000
12 months	200 000	200 000

Table 1: **Vitamin A supplementation schedule**

	Samples
Mothers	
At delivery	10 mL venous blood
2 months	5 mL blood
Monthly to 6 months	10 mL breastmilk
Mother and infant	
At birth, 2, 5, and 12 months	Nasopharyngeal swab
Infants	
At birth	5 mL cord blood
At 2, 5, 9, and 12 months	3·5 mL venous blood
At 2, 5, 7, 9, and 12 months	LMR, UBT, and stool samples
Monthly	Anthropometry
Twice a week to 12 months	Morbidity questionnaire

LMR=lactulose to mannitol ratio. UBT=urea breath test.

Table 2: **Tests and sample collection**

Data collection and procedures

All mother–infant pairs were analysed at birth, 2, 5, 7, 9, and 12 months postpartum. Timely collection of samples at delivery in the participants' homes was done by close liaison with the village traditional birth attendants, except when mothers had been referred for delivery elsewhere. Subsequent samples were obtained at the main study centre (table 2). Plasma was separated within 2 h and stored at –80ºC. Participants were monitored daily for the first 3 days after every supplementation, and then twice a week at home to assess morbidity. The monthly infant anthropometry, twice a week morbidity questionnaires, saliva and stool collections were all done during these community visits. Children who were unwell and feverish were taken to the study centre for assessment by a paediatrician.

Primary endpoints were: maternal and infant plasma vitamin A concentrations, reduction of infant *Helicobacter pylori* infection and nasopharyngeal pneumococcal carriage, and improvement of infant gut epithelial integrity.

We also measured secondary endpoints: infant growth and morbidity, breastmilk levels of vitamin A and mammary epithelial integrity, and measures of cellular immunity.

For diagnosis of nasopharyngeal carriage of *Steptococcus pneumoniae*, nasopharyngeal calcium alginate swabs (Fisherbrand, Fisher Scientific, Pittsburg, PA, USA) were immersed in skimmed milk tryptone–glucose–glycerin transport medium and stored at –80ºC before culture by standard techniques.[12]

For *Helicobacter pylori* diagnosis, breath samples were obtained with a facemask and expired air reservoir bag, at baseline and 30 min after dispensing 10 mg/kg ^{13}C-urea solution (99% atom excess, Cambridge Isotopes, MA, USA). Breath samples were transported to the MRC Human Nutrition Research in Cambridge, UK, for analysis. Isotope enrichment was analysed with continuous flow isotope ratio mass spectrometry (AP2003 IRMS, Analytical Precision Ltd, Northwich, Cheshire, UK). Diagnostic cutoff values were based on research within this cohort that achieved the best sensitivity and specificity against faecal antigen assays (unpublished).

For gut integrity assessment, infants' urine was obtained for 5 h after dosing with 200 mg/kg lactulose and 50 mg/kg mannitol. Lactulose, lactose, mannitol, and creatinine were assayed on a clinical chemistry analyzer (Roche/Hitachi 912, Hoffmann La Roche, Basel, Switzerland) with Lunn's method.[13] Here, we present only the lactulose to mannitol ratio, and use a normal cutoff of 0·30, which is derived from the mean plus two SD from a study of infants in UK.[14]

To assess the concentration of Na^+, K^+ , and vitamin A, breastmilk was obtained from both breasts by maternal manual expression. Na^+ and K^+ concentrations were measured in whole milk by flame photometry on a digital flame photometer (IL 943, Analytical Instruments, LLC, USA) with Filteau's method.[15] Vitamin A analysis was done by high performance liquid chromatography

	High dose	Standard WHO dose	Difference (95% CI)
Male	49	51	..
Female	50	47	..
Weight (kg)	2·98 (0·40)	3·03 (0·35)	−0·05 (−3·38 to 3·28)
Length (cm)	48·9 (2·0)	48·9 (2·0)	0·0 (0·0 to 0·0)
MUAC (cm)	9·80 (0·88)	9·87 (0·76)	−0·07 (−0·31 to 0·17)
Head circumference (cm)	34·5 (1·2)	34·6 (1·2)	−0·1 (−0·4 to 0·2)
Weight-for-age (*Z* score)	−1·09 (0·88)	−0·98 (0·78)	−0·11 (−0·35 to 0·13)
Weight-for-length (*Z* score)	−0·69 (1·18)	−0·47 (0·99)	−0·22 (−0·53 to 0·09)
Length-for-age (*Z* score)	−0·89 (0·99)	−0·90 (1·02)	−0·01 (−0·28 to 0·26)
Vitamin A (µmol/L)*	0·69 (0·20)	0·67 (0·21)	0·02 (−0·04 to 0·08)
Hb (g/L)*	135·2 (21·8)	134·6 (25·7)	0·06 (−0·63 to 0·75)
CRP (mg/L)*	0·36 (0·55)	0·37 (0·37)	−0·01 (−0·14 to 0·13)
AGP (g/L)*	0·18 (0·12)	0·20 (0·12)	−0·20 (−0·05 to 0·02)
Neonatal nasopharyngeal carriage (%) positive	1 (1)	2 (2)	−1·0 (−4·5 to 2·5)

Data are means (SD) or numbers (%). MUAC=middle–upper arm circumference. Hb=haemoglobin. CRP=C-reactive protein. AGP=α1-acid glycoprotein. *Cord-blood measurements.

Table 3: **Baseline characteristics of study infants**

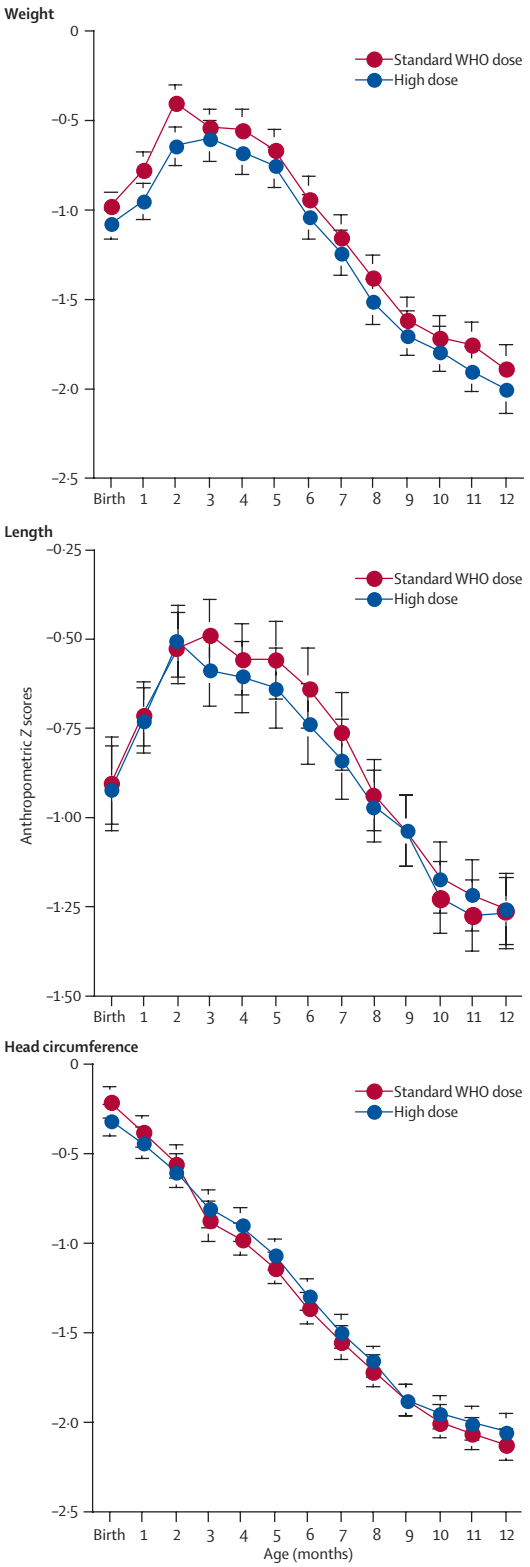

Figure 2: **Anthropometric status during infancy**
Anthropometric *Z* scores are expressed relative to the WHO standards. Error bars=SEM.

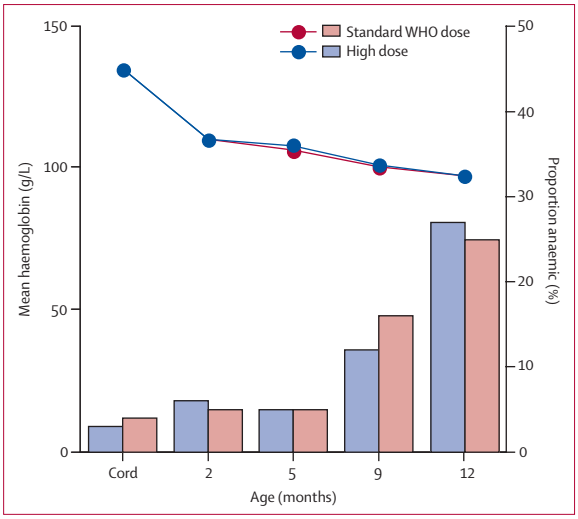

Figure 3: **Haematological status during infancy**
Lines show mean haemoglobin concentrations. Average SEM=1·3 g/L. Columns show proportion of infants with less than 90 g/L haemoglobin.

(Millipore-Waters, UK) with Jewell's method.[16] Inadequate levels of vitamin A were defined, in line with the WHO cutoff, as less than 1·05 μmol/L.[17]

Plasma retinol and β-carotene were simultaneously analysed by high performance liquid chromatography (Beckman Coulter, UK) with a modified version of Thurnham's method.[18] C-reactive protein and α1-acid glycoprotein were measured by immunoturbidimetry (Hitachi 912, Hoffmann La Roche, Basel, Switzerland) with commercial kits for high-sensitivity C-reactive protein (Dade Behring, UK) and α1-acid glycoprotein Flex reagent cartridges (Roche Diagnostics), respectively. We calculated plasma retinol values that had been adjusted for raised acute-phase proteins, as proposed by Thurnham.[19] In the present dataset, this adjustment made little difference; therefore, we present unadjusted values. We used the IVACG threshold of less than 0·7 μmol/L for the identification of vitamin A deficiency.[7]

Haemoglobin and white cell counts were estimated in fresh blood samples with an automated analyser (Medonic CA620/530, Boule Medical AB, Stockholm, Sweden). Differential white cell counts and lymphocyte subpopulations were obtained in whole blood by flow cytometry (Becton Dickinson FacsCalibur and Flowjo Software, Treestar, OR, USA) in a subset of infants at 2 months (which represented the baseline before first high-dose vitamin A administration) and at 5 months (after the first three doses of vitamin A on the high-dose schedule).

Length ±0·5 cm, weight ±10 g, head circumference ±0·1 cm, and middle–upper arm circumference ±0·1 cm were measured by standard techniques. Results are expressed as *Z* scores versus the WHO standard, which has been calculated with Cole's method.[20]

Morbidity was assessed by a questionnaire that had been designed to give basic information about the frequency, duration, and severity of illnesses that related to mucosal infections (ie, diarrhoea and acute respiratory infection), and general health from 1 week of age. When participants travelled outside West Kiang, mothers were contacted by telephone to administer the questionnaire. The questionnaire contained specific reference to fever,

	Retinol (μmol/L)			Proportion deficient*		
	High dose	Standard WHO dose	Difference (95% CI)†	High dose	Standard WHO dose	Difference (95% CI)‡
Plasma concentrations						
Infants						
Cord blood	0·69 (0·20), 92	0·67 (0·21), 92	0·02 (−0·04 to 0·08)	55% (92)	61% (92)	−5·5 (−20·0% to 9·1%)
2 months	0·74 (0·21), 63	0·72 (0·20), 71	0·02 (−0·06 to 0·09)	44% (63)	44% (71)	0·7 (−16·4% to 18·0%)
5 months	0·84 (0·21), 72	0·83 (0·21), 76	0·01 (−0·06 to 0·08)	28% (72)	32% (76)	−3·8 (−18·8% to 11·2%)
9 months	0·85 (0·23), 78	0·85 (0·22), 75	0·00 (−0·07 to 0·08)	26% (78)	31% (75)	−5·1 (−19·6% to 9·5%)
12 months	0·87 (0·25), 64	0·94 (0·28), 64	−0·07 (−0·17 to 0·02)	23% (64)	19% (64)	4·6 (−9·7% to 19·1%)
Mothers						
2 months	1·43 (0·44), 96	1·41 (0·40), 97	0·02 (−0·10 to 0·15)	4% (96)	4% (97)	0·1 (−5·7% to 5·8%)
Breastmilk concentrations§						
1 month	2·01 (1·07), 94	1·86 (0·81), 93	0·15 (−0·13 to 0·43)	13% (94)	15% (93)	−2·3 (−12·4% to 7·8%)
2 months	1·69 (0·79), 96	1·67 (0·67), 95	0·02 (−0·19 to 0·24)	18% (96)	19% (95)	−1·2 (−12·4% to 10·0%)
3 months	1·68 (0·79), 94	1·74 (0·78), 96	−0·06 (−0·29 to 0·16)	23% (94)	19% (96)	4·6 (−7·2% to 16·5%)
4 months	1·54 (0·75), 95	1·55 (0·84), 97	−0·01 (−0·24 to 0·21)	21% (95)	32% (97)	−10·9 (−23·5% to 1·7%)
5 months	1·50 (0·80), 96	1·47 (0·85), 96	0·03 (−0·21 to 0·27)	28% (96)	37% (96)	9·4 (−22·9% to 4·1%)
6 months	1·56 (0·84), 96	1·47 (0·71), 96	0·09 (−0·14 to 0·31)	27% (96)	33% (96)	−6·2 (−19·5% to 7·0%)

Data are means (SD), numbers, or percentage (numbers). *Below IVACG cutoff of vitamin A deficiency of 0·7 μmol/L.[7] †Differences were analysed by one-way ANOVA against treatment. ‡Differences were analysed by χ^2 test. §Proportion deficient below WHO cutoff for vitamin A deficiency of 1·05 μmol/L.[17]

Table 4: **Plasma and breastmilk retinol concentrations**

	High dose	Standard WHO dose	Difference (95% CI)*
Nasopharyngeal pneumococcal carriage			
Infants			
At birth	1% (98)	2% (98)	−1·0 (−4·5% to 2·5%)
2 months	80% (98)	76% (98)	3·1 (−8·7% to 14·9%)
5 months	88% (98)	88% (98)	0·0 (−9·4% to 9·4%)
12 months	76% (98)	81% (98)	−4·1 (−15·8% to 7·6%)
Mothers			
At delivery	11% (98)	15% (98)	−4·1 (−13·8% to 5·6%)
2 months	34% (98)	15% (98)	18·4 (6·4% to 30·4%)†
5 months	24% (98)	22% (98)	2·1 (−11·3% to 15·5%)
12 months	20% (98)	22% (98)	−2·0 (−13·7% to 9·7%)
***Helicobacter pylori* infection**			
Infants			
2 months	45% (92)	40% (95)	4·6 (−9·9%, 19·0%)
5 months	30% (93)	34% (93)	−4·3 (−18·0%, 9·4%)
7 months	43% (93)	45% (96)	−1·8 (−16·2%, 12·7%)
9 months	54% (97)	55% (96)	−1·6 (−15·9%, 12·7%)
12 months	55% (92)	56% (97)	−0·3 (−14·7%, 14·2%)

Data are % carriage (n). *Differences were analysed by χ^2 test. †p=0·003.

Table 5: **Indicators of respiratory and gastric mucosal infection**

	High dose	Standard WHO dose	Difference (95% CI)
Gastrointestinal damage			
Lactulose to mannitol ratio*			
Infants			
2 months	0·190, 93	0·195, 92	−0·005 (−0·80 to 1·20)
5 months	0·188, 95	0·197, 96	−0·007 (−0·77 to 1·18)
7 months	0·276, 96	0·212, 95	0·064(−1·06to1·61)†
9 months	0·300, 93	0·286, 95	0·014 (−0·86 to 1·28)
12 months	0·311, 93	0·322, 94	−0·011 (−0·80 to 1·17)
Proportion abnormal‡§¶			
2 months	11% (94)	12% (93)	−0·8 (−10·4% to 8·0%)
5 months	12% (96)	13% (97)	−0·9 (−10·6% to 8·8%)
7 months	34% (97)	22% (97)	12·4 (−0·4% to 25·1%)
9 months	35% (97)	30% (98)	5·5 (−7·9% to 18·8%)
12 months	39% (95)	34% (95)	5·2 (−8·7% to 19·2%)
Mammary epithelial damage			
Na^+ to K^+ ratio‖			
Mothers			
1 month	0·52 (0·34), 98	0·52 (0·38), 94	0·0 (−0·10 to 0·11)
2 months	0·50 (0·29), 94	0·50 (0·43), 89	0·0 (−0·11 to 0·11)
3 months	0·41 (0·20), 92	0·43 (0·29), 91	−0·02 (−0·09 to 0·06)
4 months	0·43 (0·25), 94	0·46 (0·41), 92	−0·03 (−0·14 to 0·06)
5 months	0·39 (0·14), 96	0·41 (0·28), 95	−0·02 (−0·08 to 0·05)
6 months	0·43 (0·27), 97	0·45 (0·40), 96	−0·02 (−0·12 to 0·08)
Proportion abnormal‡¶**			
1 month	31% (98)	23% (94)	7·2 (−5·6% to 20·0%)
2 months	20% (94)	17% (89)	3·3 (−8·1% to 14·8%)
3 months	11% (92)	12% (91)	−1·2 (−10·6% to 8·2%)
4 months	11% (94)	14% (92)	−3·5 (−13·1% to 6·2%)
5 months	7% (96)	9% (95)	−2·2(−10·2% to 5·8%)
6 months	11% (97)	10% (96)	0·9 (−8·0% to 9·9%)

Data are means (SD), numbers. *Data are geometric means. Geometric means were analysed by one-way ANOVA against treatment. †p=0·014, confirmed by non-parametric (Kruskal-Wallis) analysis. ‡Data are percentage (n). §Normal cutoff (0·30) set at UK mean+2 SD from Lunn et al.[14] ¶Differences were analysed by χ^2 test. ‖Differences were analysed by one-way ANOVA against treatment. **Cutoff indicative of subclinical mastitis set at 0·60, as recommended by Filteau.[15]

Table 6: **Indicators of damage to mucosal defences**

nausea, and bulging fontanelle as possible adverse consequences of vitamin A toxicity. The questionnaire was administered 1 day before and on 3 consecutive days after dosing to diagnose any adverse events. Although mothers were the principal respondents to the questionnaire, fieldworkers examined children for general wellbeing and specifically for the identification of bulging fontanelle. Children who were unwell were taken to the clinic for further examination by a paediatrician.

Statistical analysis

Prehoc power tests based on previous studies were used to calculate the sample size needed for primary outcomes. Because of an expected 60% prevalence of *H pylori* at 9 months, detection of 20% reduction (ie, to 40%) at 5% significance and 80% power would need 94 individuals per group. Pneumococcal carriage was expected to be 80%, and this sample size would detect 18% improvement (ie, to 62%). This sample size enabled detection of an effect size of 0·41 SD for the primary and secondary outcomes that were assessed as continuous variables. To allow for dropouts, our recruitment target was set at 110 individuals per group. Posthoc analysis with the actual variance figures that were obtained in this study showed the possibility to detect these differences at 5% significance and 80% power: 20% of *H pylori* prevalence, 18% of pneumococcal carriage, 0·08 µmol/L of plasma retinol, and 17% of abnormal lactulose to mannitol ratio.

Data were double-entered into a customised Microsoft Access database and were analysed with Stata version 9 (StataCorp LP, TX, USA). Binary outcomes (vitamin A deficiency, pneumococcal carriage, *H pylori* infection, increased acute-phase protein concentration, and damaged infant gastrointestinal and maternal mammary epithelium) were compared between treatment groups by Pearson's χ^2 test. Means of continuous variables were compared with ANOVA of the untransformed variable, except in the case of lactulose to mannitol ratios, for which the analysis was done in the logarithm. Severity of infection was measured as duration of episodes of diarrhoea, vomiting, fever, cough, and other illnesses. Stools per episode and bloody stools were also analysed for diarrhoea and vomiting per episode of vomiting. Visits to the clinic (both referrals by the field team and spontaneous presentations) were also analysed. To eliminate non-serious referrals, we only counted those individuals to whom a treatment was prescribed. Multilevel

modelling was used to test the effects of the treatment, with episode as the unit of analysis, and adjustment for sex and season. No allowance was made for multiple testing, although the few results that were significant (seven of ≥180) are not more than might be expected from the number of independent tests done. Regression models controlling for the sex of the baby or season of birth gave identical conclusions with respect to the effects of supplementation, and are not presented here.

Role of the funding source

The UK Medical Research Council funded the study, and laboratory analyses in Coleraine were supported by BASF Aktiengesellschaft (Ludwigshafen, Germany). The funding sources had no role in the study design, collection, analysis and interpretation of the data, or decision to publish. All authors had full access to the data.

Results

Of the 220 mother–infant pairs originally recruited, 197 completed the full protocol. The distribution and reasons for dropouts are shown in figure 1. Table 3 shows baseline characteristics of the infants. Inspection of adverse events yielded no episodes that indicated adverse reactions to vitamin A. There were two neonatal deaths in each group, two infant deaths in the high-dose group, and none in the WHO-dose group, but this difference was not significant. Figure 2 illustrates the growth of infants that is very poor in this community, in which babies are born small compared with WHO reference standards, show some catch-up growth when fully breastfed for the first few months of life, but then their nutritional status severely deteriorates to reach average values of –2·0 *Z* scores for weight, –1·25 for length, and –2·1 for head circumference. Apart from a transient difference in length between the treatment groups at 6 months (in favour of WHO dose), there was no detectable effect of the supplementation regimen on growth.

Haematological status in the two groups was also similar to the pattern generally seen in the Gambia, with a steady fall in mean haemoglobin from 110 g/L at 2 months to 97 g/L at 12 months (figure 3). The proportion of infants with less than 90 g/L haemoglobin rose from 5% at 2 months to 25% at 12 months (figure 3), and the proportion of infants with less than 110 g/L haemoglobin rose from 40% at 2 months to 85% at 12 months (data not shown). There were no differences between treatment groups.

Maternal plasma retinol at 2 months postpartum averaged about 1·45 µmol/L, with only 4% of women in each group falling below the deficiency threshold of 0·7 µmol/L (table 4). In infants, mean plasma retinol rose gradually from about 0·70 µmol/L in cord blood to 1·0 µmol/L by the end of the study. The proportion of infants who were classified as vitamin A deficient declined from about 60% to 20%. The two groups responded almost identically. At 1 month postpartum, there was a non-significant trend towards higher levels of breastmilk retinol in the high-dose group than in the WHO group (table 4). At 1 month postpartum, about 15% of values fell below the WHO cutoff for vitamin A deficiency (1·05 µmol/L) and this rose to about 30% at 6 months postpartum.

The indicators of mucosal defences also revealed no benefit of high-dose over WHO regimen. Presumptive rates of *H pylori* infection, which were detected by the labelled urea breath test, were 40–45% in 2-month-old infants, then fell to 30–35% in 5-month-old infants, and rose again to about 56% in 1-year-old infants (table 5). The urinary lactulose to mannitol ratio, which was used to test intestinal permeability, rose by about 50% from age 2 months to 1 year (table 6). At 7 months, the high-dose group had higher lactulose to mannitol ratios than did the WHO group (p=0·014).

	High dose	Standard WHO dose	Difference (95% CI)
Total white cells (×10³/mL)			
2 months	9·14 (2·60), 40	8·14 (3·20), 36	1·00 (–0·35 to 2·34)
5 months	9·82 (3·40), 38	9·91 (3·65), 36	–0·09 (–1·73 to 1·55)
Lymphocytes*			
2 months	74% (9·35), 41	74% (9·86), 37	0·70 (–3·64 to 5·09)
5 months	74% (8·89), 38	75% (12·02), 37	–0·31 (–5·20 to 4·59)
Monocytes*			
2 months	2% (1·73), 41	2% (1·46), 37	0·26 (–0·47 to 0·98)
5 months	1% (0·80), 38	1% (2·32), 37	–0·50 (–1·31 to 0·30)
Neutrophils*			
2 months	22% (9·75), 41	23% (9·02), 37	–1·19 (–5·44 to 3·06)
5 months	23% (8·70), 38	22% (11·34), 37	1·26 (–3·41 to 5·94)
T cells†			
2 months	58% (8·42), 42	62% (8·34), 39	–3·49 (–7·21 to 0·24)
5 months	58% (8·68), 41	58% (9·39), 42	–0·62 (–4·59 to 3·35)
B cells†			
2 months	30% (9·74), 42	28% (8·84), 39	2·21 (–2·01 to 6·24)
5 months	34% (9·89), 41	33% (9·92), 42	1·45 (–2·89 to 5·80)
Natural killer cells†‡			
2 months	9% (4·87), 41	8% (4·54), 39	0·95 (–1·16 to 3·05)
5 months	7% (3·41), 40	8% (4·02), 42	–0·47 (–2·11 to 1·17)
CD4⁺ T cells§			
2 months	34% (6·99), 42	38% (9·66), 39	–1·95 (–7·35 to 3·26)
5 months	32% (10·34), 41	35% (7·68), 42	–3·98 (–9·90 to 1·95)
CD8⁺ T cells§			
2 months	22% (8·05), 42	22% (8·47), 39	2·82 (–2·56 to 8·20)
5 months	21% (6·17), 41	21% (7·01), 42	0·87 (–3·48 to 5·21)
CD4⁺ T cells to CD8⁺ T cells ratio§			
2 months	2% (0·76), 42	2% (1·17), 39	–0·33 (–0·77 to 0·11)
5 months	1·92 (1·32), 41	2% (1·04), 42	0·25 (–0·80 to 0·30)

Data are means (SD), numbers. *Expressed as percentage of all white blood cells. †Expressed as percentage of all lymphocytes. ‡Also calculated after log transformation to account for skewness. There was no significant difference between treatment groups. §Expressed as percentage of all T cells.

Table 7: **Lymphocyte counts**

	Diarrhoea		Vomiting		Fever		Cough		Other		Total	
	High dose	Standard WHO dose	High dose	Standard WHO dose	High dose	Standard WHO dose	High dose	Standard WHO dose	High dose	Standard WHO dose	High dose	Standard WHO dose
1–3 months	3·8 (6·8)	4·5 (6·9)	1·3 (2·8)	1·8 (3·4)	5·5 (8·7)	5·7 (7·1)	9·8 (13·8)	10·8 (12·2)	9·1 (16·0)	9·0 (13·7)	29·5 (37·0)	31·8 (33·6)
Difference (95% CI)	−0·7 (−2·7 to 1·2)		−0·5 (−1·4 to 0·4)		0·2 (−2·4 to 2·1)		−1·0 (−4·7 to 2·7)		0·1 (−4·2 to 4·3)		−2·3 (−12·4 to 7·8)	
4–6 months	2·8 (4·8)	3·2 (5·1)	1·2 (3·0)	1·8 (5·0)	7·1 (8·3)	7·2 (9·0)	11·3 (12·7)	13·2 (16·5)	8·2 (11·6)	9·2 (14·7)	30·6 (32·3)	34·6 (36·4)
Difference (95% CI)	−0·4 (−1·9 to 1·0)		0·6 (−1·8 to 0·6)		0·1 (−2·5 to 2·4)		−1·9 (−6·1 to 2·3)		−1·0 (−4·8 to 2·8)		4·0 (−13·8 to 5·8)	
7–9 months	4·8 (7·4)	3·5 (5·1)	1·7 (3·3)	1·6 (3·3)	9·8 (9·5)	9·3 (9·9)	14·0 (13·8)	14·2 (15·7)	9·5 (11·6)	11·2 (13·3)	39·7 (37·5)	39·9 (37·1)
Difference (95% CI)	1·3 (−0·5 to 3·1)		0·1 (−0·9 to 1·0)		0·5 (−2·3 to 3·2)		−0·2 (−4·5 to 3·9)		−1·7 (−5·3 to 1·8)		−0·2 (−10·8 to 10·5)	
10–12 months	2·2 (3·8)	2·0 (4·4)	0·8 (2·1)	1·3 (3·8)	6·0 (6·8)	6·5 (8·5)	10·7 (14·1)	11·0 (14·4)	6·8 (12·6)	10·9 (15·0)	26·4 (31·8)	31·8 (36·8)
Difference (95% CI)	0·2 (−1·1 to 1·3)		−0·5 (−1·3 to 0·4)		−0·5 (−2·7 to 1·7)		−0·3 (−4·4 to 3·7)		−4·1 (−8·1 to −0·2)*		−5·2 (−15·2 to 4·4)	

Data are means (SD). Infant morbidity rates were assessed by active surveillance twice a week. Data are average days during which infants had symptoms over a period of 90 days. *p=0·0038.

Table 8: **Morbidity rates of study infants**

Rates of pneumococcal carriage varied greatly with age, and between mothers and their infants (table 5). At birth, only three babies (1–2%) tested positive. This rate increased to about 80% at 2 months and thereafter, with no differences between the groups. Carriage rates in mothers increased from 10–15% at delivery to around 25% between 2–12 months postpartum. At 2 months postpartum, the carriage rate in the WHO group was lower than that in the high-dose group (15·3% [95% CI 8·8–24·0] *vs* 33·7% [95% CI 24·4–43·9]; p=0·003).

Breastmilk Na^+ to K^+ ratios decreased slightly as lactation progressed (table 6). With a value of 0·6 to indicate a permeable epithelium, which is consistent with subclinical mastitis, the number of mothers affected by mammary epithelial damage fell from 20–30% 1 month postpartum to 10% 6 months postpartum. No detectable difference between the high-dose and the WHO group was present.

Table 7 summarises white cell counts and differential analysis in the subset of infants for whom these endpoints were measured. None of the treatment effects was significant.

Maternal reports of their infants' symptoms were similar between the groups and show a substantial burden of disease, as would be expected in this part of the world (table 8). At 10–12 months, the WHO group reported a greater number of symptoms (classed as other in table 8) than did the high-dose group (p=0·004). These symptoms included rashes and other minor ailments. However, when they were analysed by inclusion of measures of duration and severity (see Methods), there were no significant treatment differences. The proportion of infants in the high-dose group during the first 6 months of life and for the whole of the first year who attended the paediatric clinic, either as self-referrals or referred by study fieldworkers, was greater than it was in the WHO group: 24% *vs* 13% at 1–3 months (p=0·049); 29% *vs* 15% at 4–6 months (p=0·018); 31% *vs* 28% at 7–9 months (p=0·56); 22% *vs* 16% at 10–12 months (p=0·29); 49% *vs* 32% at 1–12 months (p=0·016). These differences were not significant for any individual diagnosis, possibly due to the low numbers.

The concentrations of acute-phase markers C-reactive protein and α1-acid glycoprotein were initially low and rose sharply during infancy (table 9). With the use of standard (adult) cutoffs from healthy individuals, over a

	High dose	Standard WHO dose	Difference (95% CI)*
C-reactive protein†‡			
Cord	0·36 (0·55), 93	0·37 (0·37), 93	−0·01 (−0·14 to 0·13)
2 months	1·49 (2·91), 78	2·31 (4·39), 79	−0·92 (−2·00 to 0·37)
5 months	5·23 (10·33), 85	3·67 (6·67), 86	1·56 (−1·10 to 4·23)
9 months	6·07 (13·90), 85	9·49 (30·45), 82	−3·42 (−10·79 to 3·95)
12 months	10·30 (26·94), 65	8·12 (13·87), 69	2·18 (−5·29 to 9·65)
Proportion abnormal§¶			
Cord	0% (98)	0% (94)	0·0 (0·0% to 0·0%)
2 months	5% (94)	10% (89)	−5·0 (−13·4% to 3·4%)
5 months	23% (92)	16% (91)	7·2 (−4·9% to 19·4%)
9 months	32% (94)	34% (92)	−2·3 (−16·9% to 12·2%)
12 months	31% (96)	36% (95)	−5·4 (−21·7% to 10·8%)
α1-acid glycoprotein‖			
Cord	0·18 (0·12), 93	0·20 (0·12), 93	−0·02 (−0·05 to 0·02)
2 months	0·72 (0·28), 86	0·72 (0·32), 88	0·00 (−0·09 to 0·09)
5 months	1·02 (0·36), 91	0·99 (0·38), 91	0·02 (−0·09 to 0·13)
9 months	1·22 (0·45), 94	1·28 (0·42), 91	−0·06 (−0·18 to 0·07)
12 months	1·30 (0·47), 67	1·29 (0·51), 69	0·01 (−0·15 to 0·18)
Proportion abnormal§**			
Cord	0% (98)	0% (94)	0·0 (0·0% to 0·0%)
2 months	17% (94)	18% (89)	−0·8 (−12·3% to 10·9%)
5 months	48% (92)	37% (91)	11·0 (−3·6% to 25·6%)
9 months	62% (94)	75% (92)	−13·0 (−26·6% to 0·5%)
12 months	69% (96)	71% (95)	−2·3 (−18·1% to 13·4%)

Data are means (SD), numbers. *Differences were analysed by χ^2 test. †Means are concentrations (mg/L). ‡Data are geometric means. Geometric means were analysed by one-way ANOVA against treatment. §Data are percentages (n). ¶Normal cutoff ≤5 mg/L.[19] ‖Means are concentrations (g/L). **Normal cut-off ≤1 g/L.[19]

Table 9: **Plasma acute-phase markers**

third of infants showed abnormally high C-reactive protein concentration by 12 months, and two-thirds had abnormally high α1-acid glycoprotein concentration. There were no differences between the treatment groups.

Discussion

The trial was powered to test for differential effect on a number of health-related outcomes, including growth, morbidity, acute-phase markers of subclinical infections, haematological and immune outcomes (haemoglobin and lymphocyte subpopulations), susceptibility of respiratory mucosa to colonisation and of gastric mucosa to infection, and the integrity of maternal mammary and infant gut epithelia. Overall, however, none of these outcomes had any additional benefit for the proposed high-dose regimen and, if anything, there was evidence for the contrary, especially in relation to the greater number of clinic attendances. Supplementation of vitamin A in preschool children reduces mortality in areas of vitamin A deficiency, but has variable effects on morbidity. Controversy exists about the optimum dosage; both higher[7] and lower[9,21] concentrations of vitamin A than the WHO standard have been recommended. Measurement of the dose that gives the maximum reduction of mortality is difficult and, without knowledge of the mechanisms by which reduction of mortality is achieved, needs many large-scale empirical trials. Potential adverse effects of vitamin A supplementation are difficult to predict, and surrogate predictors of mortality or morbidity might assist in tailoring supplementation to different environments. Vitamin A has a crucial role in the maintenance and repair of epithelial surfaces.[22] Therefore, we measured its effect on mucosal defences of children in whom moderate vitamin A deficiency[23] has been shown to coexist with persistent mucosal damage[24] and high levels of infections, which is consistent with impaired barrier protection and mucosal immunity.[25]

Ethical considerations precluded the use of an unsupplemented placebo group; therefore, we used a high-dose versus low-dose strategy. This strategy enabled comparison of the new early, high-dose schedule recommended by IVACG with the standard WHO schedule.

The absence of any detectable improvement of morbidity is in line with previous trials that have shown reduced mortality without any discernible effect on morbidity.[26–29] This paradox is further emphasised by the results of this trial that adds a broad range of intermediate markers that might both enhance susceptibility to infection and be affected by infections. None of these intermediate markers showed consistent evidence of any differential effect of the two dosing regimens, despite considerable statistical power to do so. Four comparisons between the high-dose and WHO group reached statistical significance at one timepoint—a favourable effect on other infections at one timepoint (10–12 months) in the high-dose group, and three possible detrimental effects of the high-dose regimen (ie, an increase in maternal pneumococcal carriage at 2 months postpartum, in the infant lactulose to mannitol ratio at 7 months, and in clinic attendances during the period before 6 months postpartum). These results show little internal consistency and might be multiple-testing artifacts because more than 180 statistical comparisons were done. However, the increase in the proportion of infants who showed abnormal lactulose to mannitol ratios between 2 and 7 months was greater in the high-dose group than in the WHO group and, although it was not significant, is worthy of note. The difference in clinic attendances could be a multiple-testing artifact, but is less easy to dismiss, and is consistent with Benn and colleagues[9] finding that higher doses of vitamin A might be associated with worse outcome. These authors have suggested that vitamin A amplifies non-specific detrimental effects of diphtheria, pertussis, and tetanus vaccination.[30,31]

The failure of difference of plasma and breastmilk retinol values to show a relation to doses is consistent with previous studies,[32] and suggests that these indicators are buffered by the normal hepatic homoeostatic regulation of retinol stores and distribution and, hence, are only effective at the extremes of vitamin A status. Nonetheless, they continue to be the indicators of vitamin A status recommended by WHO and IVACG, and help to confirm that the population we studied had moderate vitamin A deficiency, as evidenced by more than 50% prevalence of values below 0·7 μmol/L in cord blood and in early infancy. The fact that only 4% of women were recorded as deficient 2 months after receiving their postnatal doses (compared with an expected prevalence of 16%)[22] and the fact that the prevalence of deficiency gradually fell during infancy from 60% to 20% suggest that both regimens improve vitamin A status. Initial concerns about the potential toxicity of vitamin A administered to young infants on the basis of observations of bulging of the anterior fontanelle[5] have generally subsided after several trials that showed that this is a benign transient event with no long-term consequences.[6,9,33] Our active surveillance for bulging fontanelle, nausea, or irritability also failed to reveal evidence for such effects after either the high-dose administration to mothers (400 000 IU early postpartum) or to infants (50 000 IU at 6, 10, and 14 weeks with their EPI immunisations).

Our results cannot be extrapolated for populations that have more-severe grades of vitamin A deficiency, and they do not preclude the possibility of a differential effect on mortality because our study does not have the power to test for this outcome. However, the results are likely to reflect the general diet–disease conditions prevailing in much of rural sub-Saharan Africa. Because the WHO dosing schedule, which is widely used throughout the region, was already designed to achieve the desired effect on mortality, and because we could not detect any additional benefits of a higher dose of vitamin A on proximal measures of growth, health, and immunity, our trial lends support to the use of the standard WHO regimen, and encourages further investigation of the

effect of lower doses of vitamin A because half the WHO dose achieved a better outcome on mortality than did the full dose in the recent trial from Guinea-Bissau.[9]

Contributors

MKD was the principal investigator involved in all aspects of the study. DIT and CN-C participated in the design, sourcing, and randomisation of the supplements, and their laboratory staff did several of the assays. CN-C assisted with training in The Gambia. RAA and OS supervised the pneumococcal microbiological assays. JAS did the white cell determinations, and GM advised on these and other aspects of study design. SSJ did the mass spectrometric analysis of ^{13}C. AJF provided statistical support at the design and analysis stages. CPD was the senior study physician who oversaw the patients' clinical care and the adverse-event monitoring. AMP provided the idea and trial funding, and participated in all aspects apart from fieldwork.

Conflict of interest statement

We declare that we have no conflict of interest.

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