PROJECT FINAL REPORT
International Development Research Centre/Micronutrient Initiative
June 13, 2001
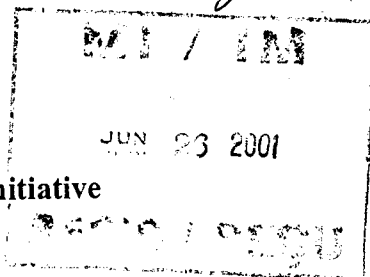
Project name:	Comparison of the effectiveness of solar-dried mangoes and high-dose vitamin A supplements in improving the vitamin A status of Gambian children
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Centre/MI file:	#5500-0003-81-300/91-0313-104

I. PROJECT OBJECTIVES

This study was designed to compare the effectiveness of food-based carotene supplements and a high-dose vitamin A supplement to improve the vitamin A status of poor Gambian preschoolers during a 4-mo period when vitamin A intake in the home diet typically was low.

The specific objectives were:

1) To measure the serum retinol and C-reactive protein (CRP) and plasma beta-carotene levels of rural Gambian children, 2-6 years of age, before, during, and after intervention.

2) To determine the intake of provitamin A and retinol-rich foods and other foods of rural young Gambian children immediately before and during the intervention period.

3) To determine the effectiveness of two diet-based interventions (solar-dried mangoes and solar-dried mangoes with fat) and a high-dose vitamin A supplement intervention in improving and maintaining the vitamin A status of a vitamin A deficient population over four months, as compared to a control group.

4) To collect morbidity data of rural Gambian children to ensure that the study groups are comparable and the effect of morbidity on vitamin A status is taken into consideration.

The hypotheses for the third objective included:

After two months of treatment:

3.1   the mean increase in serum retinol levels of the mango and fat group will be significantly higher than that of the mango only and the control groups.

3.2   the mean increase in serum retinol levels of the mango only group will be significantly higher than that of the control group.

3.3   the mean increase in serum retinol levels of the high-dose vitamin A group will be significantly higher than that of the control group.

After four months of treatment:

3.4   the mean increase in serum retinol levels of the mango and fat group will be significantly higher than that of the mango only, the high-dose vitamin A, and the control groups.

3.5   the mean increase in serum retinol levels of the mango only group will be significantly higher than that of the high-dose vitamin A and the control groups.

3.6   the mean increase in serum retinol levels of the high-dose vitamin A group will be significantly higher than that of the control group.

3.7 Use of solar-dried mangoes, solar-dried mangoes with fat, or high-dose vitamin A supplements over a four-month period will significantly decrease the percentage of the population with serum retinal levels less than 0.7 µmol/L as compared to the control group.

1. PROJECT ACCOMPLISHMENTS

Study design

This study was conducted during the dry season, November, 1998 through April, 1999 in Jiffarong, a rural farming village of 1100 residents in The Gambia, West Africa. The site was endemic with malaria and had a high prevalence of sub-clinical vitamin A deficiency (WHO 1997). There were 218 children between 2.0 to 6.9 years of age living in the village (Figure 1). Of the 218 children, 204 were measured for weight and height; 14 were not measured because of parental refusal (n=13) or child refused weighing (n=1). An additional 28 children were excluded from the study because of weight-for-height < - 2 standard deviations below the National Center for Health Statistics mean (n = 11); lack of a 3-day period free of illness during study enrollment (n = 5); parents withdrew their consent before randomization to treatment (n=8); and not recruited before the end of the enrollment period (n=4). No children were excluded because of allergy to mangoes, vitamin A supplementation within the past four months, or clinical symptoms of vitamin A deficiency.

The experimental design included the randomization of the children to one of four treatments:

- A: high-dose vitamin A, a single capsule of 200,000 IU vitamin A and 40 IU vitamin E, given at baseline (n=44)
- P: placebo, a single capsule of 40 IU vitamin E, given at baseline (n=43)
- M: mango, 75 g of dried mango per day, five d/wk for four months (n=45)
- MF: mango and fat, 75 g of dried mango and 5 g sunflower oil per day, five d/wk for four months (n=44)

Selection of mango intervention. The dose of 75 g of dried mango (296 RE) was chosen for two reasons. First, based on preliminary field work (described in the April 23, 2000 report), this dose represented the amount that young children could be expected to eat in one setting. Second, it provided 75 % of the RDA for vitamin A; we assumed that the remaining 25% would come from the children's diet.

Success of completion of interventions. All subjects in the capsule groups (A and P) were given their capsules (Sight and Life, Hoffman-La Roche, Switzerland) at the Medical Research Council's (MRC) Keneba clinic after baseline blood samples were collected. The neck of the capsule was cut and the contents squeezed into the child's mouth. We documented that the subjects swallowed the contents of the capsules and observed them for one hour after treatment. No child vomited. All children in the placebo group received a single high-dose capsule of vitamin A (200,000 IU) at the end of the study.

The subjects in the M and MF groups were given an initial mango treatment at the clinic immediately after the blood draw and then continued their supplementation in the village. All consumption was monitored and there was no sharing of mangoes among the children. The children liked the mangoes; they formed a network and would collect each other to come to the supplementation center five days per week. Those children (n= 6 M, n= 3 MF) who were absent during part of the period of supplementation were not supplemented for missed days. Ninety-nine percent of the children were supplemented at the end of each week.

Data collection. Blood samples were collected from each subject at baseline, and two and four months to measure plasma retinol, beta-carotene, and C-reactive protein. Data were collected for 16 weeks by trained field workers on daily mango supplement consumption, weekly

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assessment of home diet and morbidity (recall of daily symptoms of diarrheal and respiratory illnesses), monthly anthropometry, and socioeconomic status at the beginning and end of the study. The project results are described in reference to the four specific aims. Baseline characteristics are shown in Table 1. There were no significant differences among treatment groups in baseline characteristics.

Specific aim 1: To measure the plasma retinol, beta-carotene, and C-reactive protein (CRP) levels of rural Gambian children, 2- 6 years of age, before and after intervention.

Blood collection and analysis

Baseline blood was drawn when the child was healthy and had been free of symptoms of illness for at least three days. All 176 children were successfully bled at baseline. Eleven subjects were not bled at the 8-week visit because of refusal (n=2), unable to successfully bleed the child after three attempts (n=1), and out-migration (n=8). For the 16-week visit, 169 subjects were bled. The reasons for the seven no-bleeds included refusal (n=5), out-migration (n=1), and hospitalization of child (n=1). During each bleeding, approximately 2 ml of blood were collected in an EDTA tube by venous puncture. A blood smear was made for malarial parasites and hemoglobin levels measured by the oxyhemoglobin method (Henry 1991).

The EDTA tubes were centrifuged at 3800 revolutions per minute rpm and at least 1.6 ml of plasma were aspirated and frozen in duplicate at -80 °Celsius. The samples were protected from UV light by wrapping them in aluminum foil. Briefly, retinol and beta-carotene were extracted from plasma using the procedures of Prince et al, 1993 and Bulux et al, 1994. HPLC analysis of retinol and beta-carotene (cv within run) were carried out in duplicate in yellow light using an L-4200 single detector (Hitachi, Dansbury, Connecticut) connected to an integrator (model D-2500 chromato-integrator; Hitachi). The mobile phase consisted of 70:30:0.01 acetonitrile: methylene chloride and beta-hydroxytoluene (volume: volume: weight) at a gradient mobile phase of 1.0 ml/min. The HPLC effluent was monitored at 325 nm for retinol, 450 nm for beta-carotene and 460 nm for trans beta-apocarotenal. The total run time was 13 minutes. Quantitation was by external standardization. A standard curve for beta-apo-8 carotene was prepared from three dilutions of beta-apocarotenal. Values for both retinol and beta-carotene were corrected for recovery at 95-100 % by comparison with an internal standard of trans beta-apocarotenal. Volunteer plasma was ran each time with subject samples and NIST standards (NIST Gaithersburg, Maryland) and samples from another laboratory were used as quality control and found to be within (10 %).

C-reactive protein (CRP) assay was assayed using radioimmunodiffusion kits (The Binding Site, Birmingham, UK) and read off a linear calibration curve constructed using the neat calibrator (concentration 52 mg/L) plus two dilutions (60%, 31.2 mg/L and 10% 5.2 mg/L of the calibrator).

Analysis of Covariance was used to compare the serum retinol and beta-carotene levels between treatment groups. All comparisons controlled from CRP.

Plasma results

Beta-carotene and Vitamin A status. There were no differences in baseline, two months and first 0-2 month changes in beta-carotene levels across the four treatment groups (Table 2). At the end of the intervention, beta-carotene levels were highest in the M alone and MF groups, and lowest for the P group. Changes in beta-carotene levels between 0-4 months and 2- 4 months

differed among the groups and were highest in the M alone and MF groups. Mean plasma beta-carotene levels at the end of the study was 0.87 ± 0.6 µmol/L.

At baseline, 2.9 % of the subjects had deficient serum retinol levels (< 0.35 µmol/L), and 85.2 % had marginal levels (0.35-0.69 µmol/L; Table 1). There were no differences in baseline retinol levels between the four groups (Table 2). There was no improvement in the 2-mo retinol levels. Retinol levels significantly differed across groups at the end of the four-month intervention ($p = 0.005$). Retinol was highest for the MF and A groups, intermediate for the M only group and lowest for the P group. The 0-4 and 2-4 month changes in retinol was highest in the A group, equal between the M and MF groups and lowest in the P group. The proportion of children who had serum retinol in the normal range increased by 75% in the A group and 38% in the MF group. In the M and MF intervention groups, at the end of the intervention, subjects with marginal serum retinol levels decreased from 84.25% to 69.5% and those with normal levels increased from 14.6 % to 27 % ($p<0.05$). At the end of the intervention, some of the 176 subjects ($n=7$; 3.9%) still remained deficient and 67.6 % had marginal plasma retinol levels. The mean plasma retinol level of all subjects was (0.67 ± 0.05 µmol/L) which was under the normal cut-off level of vitamin A.

C-reactive protein levels as an indicator of illness. There were no group differences in the prevalence of acute infection throughout the study as documented by elevated CRP levels (>5.2 mg/dl; Table 1). Acute illness as defined by elevated CRP levels affected more than one-third of the subjects at baseline and between 7-14.6% at the other two time points. There were no group differences in the prevalence of acute infection throughout the study as documented by elevated CRP levels. Acute illness affected over one-third children at baseline and only 18 subjects (10.2%) at the end of the study.

Specific aim 2. To determine the intake of provitamin A and retinol-rich foods and other foods of rural young Gambian children before and during the intervention period.

Dietary intake data collection and analysis

A food frequency questionnaire was used to collect dietary data at baseline and on a weekly basis for all of the 176 subjects, including 15 subjects who had moved out of the village. Interviews were conducted at homes in which the mother or child's caretaker was asked if the child had consumed specific vitamin A, beta-carotene-rich, and fat foods. The frequency (never, occasionally, once per week, 2-3 times a week, once a day, several times a day) and the quantity of food (small, medium, or large serving size) served to the child were recorded. A complementary study in a neighboring village documented actual weights of the serving sizes for the foods on the questionnaire for each age of child (data not shown).

Participants for the complementary study were chosen by systematic sampling in which every other compound was selected. In the selected compounds, households with 2-6 year olds were identified and mothers were asked to participate. Twenty-five mothers consented to participate. The mothers estimated small, medium, and large servings of commonly consumed foods. Serving sizes were given by measuring per spoonful, calabash, cup, handful, or as slices, pieces or whole, and estimated for different age groups. The questionnaire documented the serving sizes of preformed vitamin A-rich (liver, chicken, lamb, meat, kidney, fish, eggs, fresh milk, sour milk), beta-carotene-rich (palm oil, fresh chilli, tomatoes, paw paw, guava, cashew, mangoes, dark green leafy vegetables {morongo, jabanduro, baobab, kucha}, sweet potato, maize, carrots), and high fat (groundnut oil, peanut butter, pancake) foods that were commonly consumed

in this region. These foods covered 95% of vitamin A, beta-carotene, and fat intake in this population (Bates *et al.* 1984).

The nutrient composition (retinol, beta-carotene and fat) of the most commonly encountered rural Gambian foods, such as cooked cereal foods and sauces, main vegetables, fruits, fish, among others were derived from analyses of raw and cooked foods from that region and contained in MRC nutrient composition database (McCrae and Paul, 1998).

To determine the vitamin A intake of the subjects, the retinol equivalents (RE) of preformed-vitamin A and beta-carotene foods consumed were calculated. The retinol equivalents of preformed vitamin A was determined by multiplying the frequency of food consumed (f) by amount of food consumed in grams (g) and retinol/beta-carotene (c) content in RE/g of the food item (f*g*c). The RE for beta-carotene was derived by using the above formula and dividing by 6 (assuming 1 RE is equivalent to 6 µg trans β-carotene, FAO/WHO, 1988). The food intake of preformed vitamin A, beta-carotene and fat was calculated per average daily, weekly and monthly intakes.

Diet results

Primary food sources. The primary food sources that were high in retinol, beta-carotene and fat are presented in Table 3. Retinol-rich foods included fish, milk, eggs and meats and stews or sauces. Although they served as the major source of retinol for the diet, they were not frequently consumed and, when consumed, they were eaten in small quantities. The children consumed baobab, peanut butter sauce, durango, large amounts of green leaves from leaves and kucha, which were important sources of beta-carotene from the diet. However, palm oil because of its high beta-carotene content was the largest contributor to the total beta-carotene intake of the subjects. Palm oil was consumed infrequently and in small amounts. Fat intake was not derived from traditional high-fat foods (butter, milk, cheese) but from leaves, tomatoes, fresh chilli, peanuts, and groundnut oil. Groundnut oil was consumed mostly during special occasions (naming and wedding ceremonies).

Contribution of home diet. There was seasonal variation in food availability. Maize, rice, millet, and groundnuts were generally available from November- January during the harvest season. Three to four months after the harvest season, leaves (kucha, baobab), peanut butter sauce, fish, small amounts of palm oil and cereal (findi) were mostly consumed with rice which was purchased. Food intake generally was higher during months one and two of the study than months three and four when food supply was low. The children consumed mostly cereal foods, rice, sometimes served with fish, very little fruits and vegetables, and little vitamin A-rich foods. A thin cereal porridge (mono) was served or leftovers of the previous evening's meal. The children did not replace their normal breakfast with the mango supplement as determined through the weekly food frequency.

The mean daily dietary intakes of retinol, beta-carotene and fat from the home diet are presented in Table 4. The mean daily retinol intake during the first two months and the last two months of the study were not significantly different. The mean intake of the subjects remained low throughout the study (118.4 ± 52 RE). Beta-carotene intake at the beginning of the study was low and remained low throughout the duration of the study (35.7 ± 10 RE). From 0-4 months, the total RE intake from preformed vitamin A and beta-carotene was 154.1 RE which was far below the recommendations of the FAO/WHO of 400 RE/day for 2-7 year-olds and 38.5 % of the RDA for vitamin A. Daily fat intake was low throughout the study. The daily fat intake

was almost 3 g, far less than the minimum daily fat intake of 5-10 g that is recommended for optimal vitamin A absorption and utilization (Jayaranjan *et al* 1988).

Nutrient contribution from supplement. The mango supplement contained 395.1 RE/100g (rehydrated samples ranged from 347.2 to 463.8 RE/100 g). There was only a minor difference in the mean distribution of total grams of mangoes eaten by the M alone (7714 ± 1904 g; 30,478 RE) and MF groups (7499 ± 2458 g; 29,629 RE). This intake averaged about 95.1 g of rehydrated mango per supplementation day or about 375.7 RE/day supplemented. Added to the home diet RE contribution of 154.1 RE, children in the M and MF groups consumed about 529.8 RE, or 132% of the RDA on the supplemented days. On average over the four-month period, total RE intakes for the M and MF groups were 259.9 RE from the supplement and a total of 414.0 RE per day (103% of the RDA). This contrasted to the P group that had only 154.1 RE per day, or 38.5% of the RDA. The A group received 200,000 IU (60,000 RE) of retinol at baseline. This is about twice the amount of RE received from the mango supplement received (approximately a total of 30,054 RE over four months).

Specific aim 3: To determine the effectiveness of two diet-based interventions and a high-dose vitamin A supplement intervention in improving and maintaining the vitamin A status of a vitamin A deficient population over four months, as compared to a control group.

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Specific aim 4: To collect morbidity data of rural Gambian children to ensure that group comparablilty and the effect of morbidity on vitamin A status is taken into consideration.

Statistical analysis

Study indicators of plasma vitamin A and beta-carotene levels included dietary intakes, nutritional status, demographic and socioeconomic status (SES), and infection. Spearman's rank correlation coefficient were computed to identify strong correlations between independent variables that could cause problems of multicollinearity in the analyses. Variables that were highly correlated were not put in the model together. Simple linear regression was used to assess the relationship between the independent variables and 4-mo plasma retinol and beta-carotene levels. Then, multiple linear regression models were run separately for plasma retinol and beta-carotene. Plasma retinol levels were dichotomized at the cutoff of $< $ or ≥ 0.7 μmol/L. A logistic model was run to predict low plasma retinol levels with the above mentioned independent variables.

Determinants of plasma retinol and beta-carotene

General sample characteristics. The average age of the subjects was 4.7 ± 1.7 years; 52.8 % were 2-4 years and 47.2 % were 5-7 years old. Females made up slightly more than half (52.3 %) of the subjects.

Anthropometry. At baseline, the average weight and height of the subjects were 14.3 ± 3.6 kg and 99.4 ± 12.5 cm, respectively (Table 1). The enrolled children were not malnourished (wasted) by weight-for-height (W/H) z-scores (mean z-score was -0.87 ± 0.6). About one-third (33.5 %) of the children were stunted (height-for-age $< $ -2 z-score), indicating long-term nutritional deficiencies. At four months, the average child's weight increased to 15.2 ± 3.3 kg and average height to 102.4 ± 11.8 cm. The average W/H and height-for-age (H/A) Z-scores were -0.91 ± 0.8 and -1.51 ± 0.9, respectively. The number of children wasted increased to 6.8 % (n=12) and the percentage stunted remained about the same (32.4 %). The increase in wasted children was due in part to decreased intake of staple foods and low household food supply.

Demographic and socioeconomic status (SES). The families were large with households of 10-20 people who ate together and 6-8 people sleeping in the same room. Polygamy was widely practiced with men often marrying 3-4 wives. Each wife had about four children. People had little or no education, usually stopping at the elementary school level. People owned few possessions such as farm animals, goats, cows, horses sheep, and farming implements (hoe, carts, and ploughs).

More than half of the subjects had a socioeconomic score of four (4) based on a 0-9 scale. There was a correlation between some of the SES indicator variables. Mother's score was correlated with possession score ($r_s = 0.19$, p= 0.015) indicating that mothers who were better-off (had gold earrings, jewelry) acquired more beds, radio, watch, and cassette players. Animal score was also strongly correlated (r_s =0.49 p<0.0001) signifying that families with more animals had more possessions. Families who sold more bags of crops were more likely to have many animals as indicated by the correlation ($r_s = 0.35$, p<0.0001) between animal score and spending scores.

Health status. Over the four months of the study, subjects reported a mean total number of 14.3 ± 15.8 sick days as assessed by mother's recall. A total of 79 subjects (44.9 %) made at least one visit to the MRC outpatient clinic for treatment due to illness during the study. Malaria was diagnosed by the presence of plasmodium (malarial parasites) and clinical signs and symptoms were in 28.4 % of the subjects at the beginning of the study. Malaria was found in only four subjects at four months (one in mango, one in mango and fat, and two in vitamin A) which coincided with the end of the malarial season. Acute illness was defined by CRP levels >5 mg/dl and affected more than one-third of the subjects at baseline and between 7-14.6% at the other two time points.

There was no difference between treatment groups in the distribution of illness as determined by mother's recall of illness/symptoms or by the number of sick children who were brought into the clinic for treatment. Over the four months of the study, subjects reported a mean total number of 14.3 ± 15.8 sick days as assessed by mother's recall. Forty-five percent of the subjects were brought into the clinic for treatment due to illness. More than half of the subjects in the A (50 %) and P (55 %) groups were seen at the clinic compared to that of M (36 %) and MF (39 %) groups. Malaria was more prevalent at baseline than throughout the rest of the study and was diagnosed by the presence of plasmodium (malarial parasites) and clinical signs and symptoms in 28.4 % of the subjects.

Regression modeling

The following explanatory variables were included in the initial model to predict plasma retinol: treatment group, 4-mo W/H and H/A Z-scores and Z-score changes, age, sex, number of clinic visits, CRP levels, number of malarial parasites, plasma beta-carotene, vitamin A, beta-carotene and fat intakes, and SES score. Dummy variables were created for group treatment (high-dose vitamin A, yes/no), number of malarial parasites (< 1, >1), clinic visit (= 0, >0), and gender. Total CRP and fat and beta-carotene intakes were log transformed to obtain a linear relationship with plasma retinol levels. Both fat and beta-carotene variables included both the nutrient from the supplement and home diet. The best predictive model was obtained and the residuals were plotted against predicted values to determine the adequacy of the model. The same variables, excluding plasma beta-carotene levels, were used in a regression model to predict 4-mo plasma beta-carotene.

Few variables were significant predictors of either retinol or beta-carotene plasma levels. The final model for plasma retinol included group treatment, log of C-reactive protein, and log of

beta-carotene as significant predictors. For this analysis, the final model is shown in Table 5. An increase in plasma retinol was associated with having received the high-dose vitamin A supplement and having a higher plasma beta-carotene level (which reflected beta-carotene intake). Plasma retinol was negatively associated with infection, as measured by C-reactive protein. The model only was able to explain 18% of the variance in plasma retinol levels. Logistic regression analysis with a plasma retinol cutoff of 0.7 µmol (classifying subjects as marginally deficient) had no significant predictors.

The multiple linear regression model for plasma beta-carotene only retained the log of total beta-carotene intake (home diet and mango supplement) as a significant explanatory variable. Plasma beta-carotene levels increased with increased intake of beta-carotene. Fat intake was not significant, perhaps because it was highly correlated with total beta-carotene intake. Similar to retinol, only 13 % of the variance of 4-mo plasma beta-carotene levels could be explained.

In this population of marginal vitamin A deficiency, plasma levels were not predicted by individual or family characteristics. However, plasma levels responded to intake, as demonstrated by vitamin A supplementation and total beta-carotene intake, and infection. These results support the Analysis of Covariance work that demonstrated a positive effect on attained 4-mo retinol levels with vitamin A and beta-carotene supplementation.

Summary

The results of this study show insufficient micronutrient intakes among rural Gambian preschool children. Their intake of provitamin A-, retinol- and fat-rich foods were low. Most children lived primarily on a vegetarian diet, consuming cereals and little fruits and vegetables. Green leafy vegetables accounted for only 23 % of their total vitamin A intake. Although preformed vitamin A foods were infrequently consumed and only in small amounts, because of the nutrient density of the food, they provided the majority of the vitamin A intake.

Both high-dose vitamin A and mango and fat supplements were associated with marginally higher plasma retinol levels of young children at the end of the study but only the high-dose vitamin A supplement significantly increased plasma retinol levels from baseline. We detected no group differences that may be confounding the group analysis, including age, sex, infection, socioeconomic status, anthropometric status, and home diet. One explanation for the greater response with high dose vitamin A may be the total 4-mo dosage of vitamin A that was administered to the three supplement groups. The high-dose vitamin A group received about twice the total amount of retinol equivalents as the mango groups.

The mango and mango and fat groups had higher plasma beta-carotene concentrations at four months which indicated that beta-carotene was absorbed from the dried mango. However, plasma retinol levels did not correspondingly increase even among children with marginal serum retinol levels. There are several possible explanations. Infection could have impaired the children's vitamin A status by affecting conversion through impairing enzyme expression, decreasing absorption, increasing the loss of vitamin A through urine excretion, and diminishing the availability of amino acids for retinol-binding protein. In addition, other micronutrient deficiencies such as iron and zinc may exist which might minimize the ability to increase serum retinol levels. For example, zinc is a component of retinol binding protein, the transport protein for retinol. Finally, subjects had little dietary fat intake which is necessary for vitamin A absorption. The efficiency of beta-carotene conversion to retinol could have been affected by very low fat intake in the mango only subjects and still inadequate fat intake in the MF subjects.

Children increased their plasma beta-carotene levels in the mango and mango and fat groups. The limited conversion to retinol suggests that improved results may occur if the beta-carotene supplement is offered at greater than the RDA for vitamin A to compensate for low home dietary beta-carotene and fat intake and decreased beta-carotene absorption due to infection. The supplement was well accepted among children. The consumption of fruits as well as vitamin-A rich animal foods should be encouraged. In rural farming communities, domestic small animal breeding may be a means of improving socioeconomic status and increasing the consumption of vitamin A-rich foods. Planned interventions to combat vitamin A deficiency should include components to improve socioeconomic status of families as this is expected to lead to increased food availability, better health standards and improved vitamin A status.

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PRESENTATIONS
These results have been presented at the meetings listed below. The abstracts are attached.

Experimental Biology 2000. San Diego, CA.
> Drammeh BS, Marquis GS, Stephensen CB. Comparison of mangoes and vitamin A supplements to increase serum retinol in Gambian children. FASEB J 2000: ALB190.

Experimental Biology 2001. Orlando, FL.
> Drammeh B, Marquis GS, Stephensen CB, Eto I. A randomized fruit-based intervention study to improve Vitamin A status of rural Gambian children[1].

[1]Selected as finalist for the Society for International Nutrition Graduate Student Award

Three papers are being written at this time and are expected to be submitted in the next few months. Copies of the publications will be forwarded to IDRC.

COMPARISON OF MANGOES AND VITAMIN A SUPPLEMENTS TO INCREASE SERUM
RETINOL IN GAMBIAN CHILDREN. B.S. Drammeh, G.S. Marquis, C.B. Stephensen. Univ.
of Ala at B'ham, Birmingham AL 35294; Iowa State Univ., Ames, IA 50011;Western Human
Nutr. Res. Center, Univ. of Calif., Davis, CA 95616.

Carotene-rich foods can prevent vitamin A deficiency. Mangoes may be particularly useful
because β-carotene absorption is better from fruits than from vegetables. To test the effect of
mangoes on vitamin A status in a region with highly prevalent vitamin A deficiency, we
randomized 176 Gambian children aged 2-7 y to receive 75 g dried mangoes (rehydrated before
eating) plus 5 g fat (5d/wk for 4 mo) (MF), mangoes without fat (M), one 200,000 IU vitamin A
capsule (A), or one placebo capsule (P) at baseline. Blood was drawn at baseline, 2 and 4 mo.
Preliminary analysis of 2-mo retinol and beta-carotene levels (without adjustment for baseline
levels, dietary intake or infection) found no overall group differences. Mean retinol values were
very low, ranging from 0.61±0.038 (A) to 0.63±0.049 µmol/L (MF); beta-carotene levels ranged
from 0.52±0.17 (MF) to 0.57±0.33 µmol/L (M). The correlation of serum beta-carotene with
retinol differed significantly among the groups ($p<0.01$), suggesting a beneficial interaction of fat
and β-carotene on serum retinol. Further analysis is needed to clarify the effect of the
intervention. (Funded by Thrasher Research Fund, IDRC, Rockefeller Foundation, Bristol
Myers Squibb-PINS, Sight & Life)

A Randomized Fruit-based Intervention Study to Improve Vitamin A Status of Rural
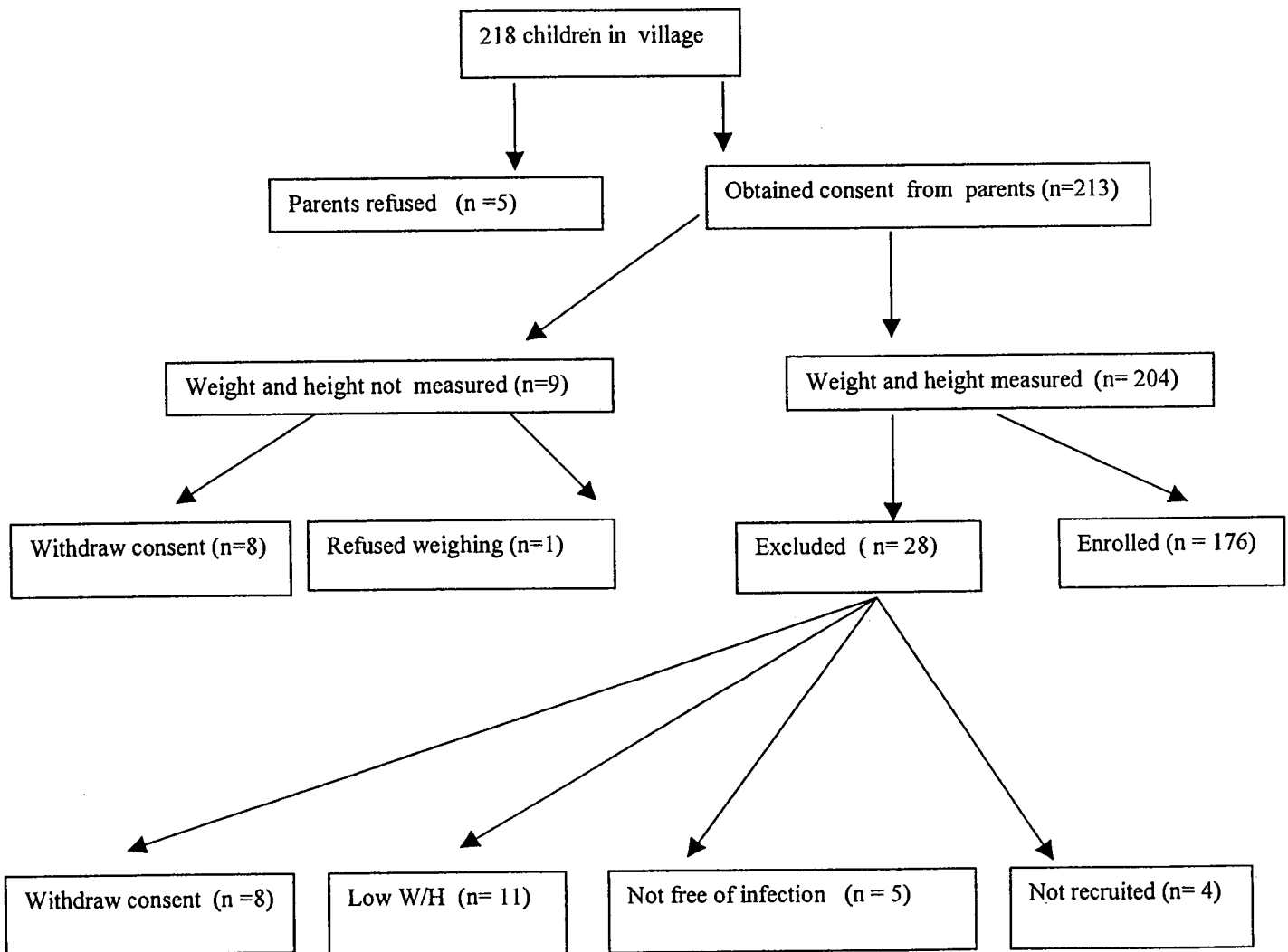Gambian Children

Bakary Drammeh[1] , Grace S Marquis[2], Charles B Stephensen[3], Isao Eto[1]
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Use of a food-based intervention to improve vitamin A status may be a sustainable approach
for regions where vitamin A deficiency is prevalent. We determined if mango supplements,
with and without fat, could improve vitamin A status when home vitamin A intake was low.
The study enrolled 176 children between 2-7 years old. Children were randomized to four
treatments; two treatments were offered 5 d/wk for 4 mo (75 g dried mangoes plus 5 g fat
[MF; n=44] and 75 g dried mangoes without fat [M; n=45]) and two treatments were given at
baseline (200,000 IU vitamin A capsule [A; n=44] or placebo capsule [P; n=43]). Blood
samples were collected at baseline, 2 mo, and 4 mo for retinol, beta-carotene, and C-reactive
protein (CRP, an indicator of infection). Data were collected over four months on supplement
consumption, home diet, morbidity, anthropometry, and family demographics and
socioeconomic status. Retinol and beta-carotene means ($\pm$ SE) were adjusted for C-reactive
protein. There were no group differences at baseline or two months. At 4 mo, serum beta-carotene
levels for the MF (1.053 ± 0.089 µmol/L) and M (0.996 ± 0.087 µmol/L) groups
were higher ($p<0.01$) than the P group (0.623 ± 0.087 µmol/L). Serum retinol levels for the
MF (0.685 ± 0.008 µmol/L) and A (0.685 ± 0.008 µmol/L) groups were also higher ($p<0.01$)
than the P group (0.650 ± 0.008 µmol/L). After 4 mo, the MF and A interventions improved
vitamin A status, as indicated by serum retinol concentration. The increases in serum retinol
were small and further studies to more directly evaluate the impact of this intervention on
vitamin A stores are warranted. (Funding: Thrasher Research Fund, Micronutrient
Initiative/IDRC, Rockefeller Foundation, Bristol Myers Squibb-PINS, Sight & Life)

This abstract was selected as a finalist for the Society for International Nutrition Student Award

Figure 1. Sample selection


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                        ┌─────────────────────────┐
                        │ 218 children in village │
                        └─────────────────────────┘
                              │              │
                              ▼              ▼
        ┌──────────────────────┐      ┌──────────────────────────────────────┐
        │ Parents refused (n=5)│      │ Obtained consent from parents (n=213)│
        └──────────────────────┘      └──────────────────────────────────────┘
                                       │                        │
                                       ▼                        ▼
        ┌──────────────────────────────────┐   ┌────────────────────────────────────┐
        │ Weight and height not measured   │   │ Weight and height measured (n= 204)│
        │ (n=9)                            │   └────────────────────────────────────┘
        └──────────────────────────────────┘      │                    │
            │                    │                 ▼                    ▼
            ▼                    ▼         ┌────────────────┐   ┌──────────────────────┐
    ┌──────────────────┐  ┌────────────────────────┐ │ Excluded (n= 28)│ │ Enrolled (n = 176)  │
    │ Withdraw consent │  │ Refused weighing (n=1) │ └────────────────┘   └──────────────────────┘
    │ (n=8)            │  └────────────────────────┘
    └──────────────────┘
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Excluded (n= 28):

| Withdraw consent (n =8) | Low W/H (n= 11) | Not free of infection (n = 5) | Not recruited (n= 4) |

Table 1. Characteristics of study population

	Study Group			
	Mango alone n=45	Mango & Fat n=44	Vitamin A n=44	Placebo n=43
Age (yr)	4.8 ± 1.7 [1]	4.6 ± 1.7	4.7 ± 1.6	4.8 ± 1.8
2-4	20 (44.4) [2]	27 (61.4)	25 (56.8)	21 (48.8)
5-7	25 (55.6)	17 (38.6)	19 (43.2)	22 (51.2)
Gender				
Male	19 (42.2)	21 (47.7)	20 (45.4)	24 (55.8)
Female	26 (57.8)	23 (52.3)	24 (54.6)	19 (44.2)
Baseline				
Weight (kg)	14.1 ± 4.1	14.0 ± 3.2	14.6 ± 2.9	14.7 ± 4.1
Height (cm)	99.6 ±13.7	98.4 ±12.2	99.5 ±11.4	100.3 ±12.7
SES[3]	4.7 ± 2.1	4.2 ± 2.1	4.8 ± 1.8	4.5 ± 1.7
Retinol baseline				
Deficient	0 (0)	1 (2.3)	1 (2.3)	3 (7.0)
Low	40 (88.9)	35 (79.6)	39 (88.6)	36 (83.7)
Normal	5 (11.1)	8 (18.1)	4 (9.1)	4 (9.3)
Any malarial parasites at baseline [4]	12 (26.7)	12 (27.3)	13 (29.6)	13 (30.2)
CRP level >5.2				
Baseline	15 (33.3)	18 (40.9)	18 (41.9)	14 (35.0)
At 2 months	3 (7.0)	6 (14.6)	6 (14.0)	5 (13.5)
At 4 months	5 (11.6)	4 (9.3)	6 (14.0)	3 (7.5)

[1] Mean ± SD

[2] n (%)

[3] SES: Possession of household items, domestic animals, rank of mother, house, and bags of crops sold

[4] Only two children had measurable parasites at two months (both in mango and fat group), and four children at four months (one in mango, one in mango & fat, and two in vitamin A group).

No significant difference in baseline characteristics between groups.

Table 2. Serum beta-carotene and retinol (μmol/L)
Baseline & follow-up, adjusted for CRP

| | Study Group | | | | |
	Mango alone	Mango & Fat	Vitamin A	Placebo	p
Beta-carotene					
Baseline	0.48 (0.05)	0.55 (0.05)	0.49 (0.05)	0.43 (0.05)	0.541
At 2 months	0.55 (0.04)	0.55 (0.04)	0.56 (0.04)	0.54 (0.04)	0.845
1st 2-month change	0.05 (0.05)	-0.04 (0.05)	0.06 (0.05)	0.10 (0.05)	0.295
At 4 months	1.0 (0.08)	1.1 (0.08)	0.80 (0.08)	0.60 (0.08)	0.009
2nd 2-month change	0.49 (0.08)	0.56 (0.08)	0.25 (0.07)	0.08 (0.08)	<0.001
4-month change	0.52 (0.08)	0.5 (0.08)	0.32 (0.08)	0.18 (0.08)	0.040
Retinol					
Baseline	0.63 (0.01)	0.65 (0.01)	0.62 (0.01)	0.63 (0.01)	0.282
At 2 months	0.62 (0.01)	0.63 (0.01)	0.61 (0.01)	0.62 (0.01)	0.219
1st 2-month change	-0.01 (0.01)	-0.02 (0.01)	-0.01 (0.01)	-0.01 (0.01)	0.988
At 4 months	0.67 (0.01)	0.69 (0.01)	0.69 (0.01)	0.64 (0.01)	0.005
2nd 2-month change	0.05 (0.01)	0.05 (0.01)	0.07 (0.01)	0.03 (0.01)	0.017
4-month change	0.04 (0.01)	0.04 (0.01)	0.06 (0.01)	0.02 (0.01)	0.024

Table 3. Primary food sources of retinol, beta-carotene, and fat intake from the home diet from 0-4 months (n=176)

Foods rich in macro and micronutrients		
Vitamin A	**Beta-carotene**	**Fat**
Meats/organ meats	Baobab	Leaves
Milk, as a drink	Durango	Tomatoes
Milk, porridge	Leaves	Fresh chilli
Eggs	Kucha	Peanuts
Fish	Palm oil	Groundnut oil

Table 4. Mean (± SD) daily intake of vitamin A,
beta-carotene, and fat intake from the home diet

Nutrient	Amount
Retinol (RE)	
0-2 month daily	127.3 ± 83.0
2-4 month daily	110.6 ± 53.0
0-4 month daily	118.4 ± 52.0
Beta-carotene (RE)	
0-2 month daily	35.6 ± 13.8
2-4 month daily	34.6 ± 12.0
0-4 month daily	35.7 ± 10.0
Fat (g)	
0-2 month daily	2.7 ± 0.7
2-4 month daily	2.9 ± 0.6
0-4 month daily	2.9 ± 0.5

Table 5. Regression models for plasma retinol and beta-carotene [1]

Variable	Coefficient	Std Error	P-Value	R-Square
Outcome: Plasma Retinol				
Vitamin A (1=yes; 0=no)	0.24	0.087	0.0063	
Log (C-reactive Protein)	-0.08	0.03	0.0094	
Log (plasma beta-carotene)	0.17	0.06	0.0088	0.18
Outcome: Plasma Beta-carotene				
Total dietary beta-carotene	1.57×10^{-7}	5.39×10^{-7}	0.001	0.13

[1] Only significant variables are shown. Other variables that were not significant and were removed from the model included: SES score, Wk 16 W/H Z-scores, Wk 16 H/A Z-scores, High dose vitamin A (yes/no), male (yes/no), clinic visit (< 0 or >0), log transformation of CRP, number of malarial parasites (< 1 or >1, dummy variable), dietary vitamin A, log transformation of beta-carotene (diet + mango supplement), log transformation of total fat (diet + fat supplement)