The protective effect of red palm oil in comparison with massive vitamin A dose in combating vitamin A deficiency in Orissa, India

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Vitamin A deficiency has long been a serious hazard to the world community, especially to children. The main reason for a higher incidence among children is lack of vitamin A in the diet. Carotene rich supplements with sources like Red Palm oil (RPO) could be used as a measure to combat vitamin A deficiency. This study evaluates the protective effect of RPO in comparison with massive vitamin A dose to combat vitamin A deficiency. The study was carried out for a period of three months in 36 school children. Twelve children received a massive dose (50,000 IU) of vitamin A, another twelve children received 4g of RPO containing B-carotene equivalent to 25,000 IU of vitamin A in "Besan laddu" and the remaining twelve received 8g of RPO containing B-carotene equivalent to 50,000 IU of vitamin A in "Besan laddu". Serum vitamin A levels were estimated initially, after 15 days of supplementation and 3 months after termination of supplementation. The levels were maximum 15 days after the supplementation and, though it fell by the end of 3 months, yet it was significantly higher than that of the initial levels in all the three groups. Among both the levels of RPO supplement, 8g RPO was as efficient as was a massive vitamin A dose in providing protection for three months, after cessation of supplementation.

Key words: vitamin A deficiency, Orissa India, children, red palm oil

Introduction

Vitamin A deficiency is the most widespread nutritional disorder causing blindness to the children in developing countries particularly in tropical and subtropical countries. The rational approach for prevention of such wide spread vitamin A deficiency, in our children would obviously be in the improvement of their diets[1]. Carotene rich supplements with sources like red palm oil (RPO) could be used as a measure to combat vitamin A deficiency. Studies indicated that Indian school children fed supplementary snacks prepared with RPO for 60 days had significant increases in serum retinol levels as well as an increased liver retinol store, suggesting the ready bioavailability of β-carotene from red palm oil[2].

This study proposes to evaluate the protective effect of RPO in comparison with massive vitamin A dose in combating vitamin A deficiency. It has been established that 200,000 IU of massive vitamin A dose to preschool children and 100,000 IU to school children have protective effect for 6 months[3]. Information on the protective effect of RPO, by giving a gap of three months after supplementation, was thought to be very valuable in formulating policies for combating vitamin A deficiency.

Method

Ethical consideration

Informed consent was obtained from the children's mothers and the principles required by the Helsinki declaration met. All children with a clinical diagnosis of vitamin A deficiency were offered a form of vitamin A supplementation; and long-term nutritional advice was provided to mothers and children.

Sampling

One hundred children belonging to an interior village called "Nakhaur" in Puri district, Bhubaneshwar, Orissa, were screened by a group of trained paediatricians involved in public health programmes, for clinical signs and symptoms of vitamin A deficiency. The presence or absence of clinical signs like night blindness, conjunctival or corneal xerosis and Bitot's spots, was noted. 36 school children in the age group of 7-9 years with mild to severe signs of vitamin A deficiency were selected. The 36 children were randomly assigned to three groups, each group containing 12 children.

Group I (control) was given a mega dose of vitamin A (50,000 IU) (half of 100,000 IU which is the dose given to school age children. Only 50% of the dose was given, as the study period was only 3 months).

Group II was given 4g RPO every day for 15 days containing β-carotene equivalent in toto to 25,000 IU of vitamin A in "Besan laddu" (a sweet snack made from chick pea flour, sugar and fat, in the shape of balls).

Group III was given 8g RPO every day for 15 days containing β-carotene equivalent in toto to 50,000 IU of vitamin A in "Besan laddu".

The carotenoid composition of the RPO was 550ppm.

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Clinical assessment

Children were assessed for general signs and symptoms of vitamin A deficiency especially for Bitot's spots and conjunctival xerosis, at the beginning, after 15 days supplementation and, finally, 3 months after cessation of supplementation.

Anthropometric assessment

Height, weight and mid-arm circumferences were measured at the beginning of the study period. The weight and height measurements of children were converted into weight for age, height for age and weight for height per cent of standard for each child using NCHS standards[4]. Children were classified into grades of nutritional status using Waterlow's classification[5]. Shakier and Morley cut-off points were used for the classification of mid arm circumference[6]. Thus the children were classified into four grades of nutritional status: normal, wasted, stunted and both stunted and wasted.

Dietary assessment

Mothers were interviewed by the 24 hour recall method. It was conducted four times during the study, initially, at the end of 15 days of supplementation, one month after supplementation ceased, and at the end of the study period. The nutrient content of the child's diet in terms of vitamin A was calculated using the food consumption tables for India. No advice was given to change diet during the study, although it was at the conclusion.

Assessment of serum vitamin A levels

Serum vitamin A levels of the 36 children were assessed using high performance liquid chromatography (HPLC) method[7]. The HPLC system used was the Shimadzu LC-6A, with a UV detector SPD-6A, isocratic pump LC-6A, Shimpak Column, CLC-ODS (M) C18, 25 cm x 4.6 nm, rheodyne injection valve, SCL-6A system controller and CR-6A recorder.

100 µl of blood was drawn from each child by the finger prick method to estimate serum retinol levels. This was done before supplementation, 15 days after supplementation and, finally three months after supplementation was completed.

Blood from the finger tip was allowed to run in about 3/4 mm length of a heparinised capillary of 7.5 nm length. The tubes were then centrifuged in a microhaematocrit for 5 minutes. Serum was separated and stored at -20° C for estimation of vitamin A.

Serum, ethanol and an internal standard (retinyl acetate) were mixed vigorously on a vortex mixer. HPLC grade hexane was added and the contents again mixed on a vortex mixer, until the bottom layer was thoroughly extracted. The contents were centrifuged at 2000 rpm for 5 minutes, the upper hexane layer transferred to a small test tube and evaporated under nitrogen. The remaining lipid residue was dissolved in methanol. An aliquot of 20 µl of the solution was injected onto the HPLC column. Plasma retinol concentrations were calculated from the standard curve after correction of per cent loss using the internal standard.

Statistical analysis

One way analysis of variance was conducted to assess differences between (a) three groups at three time points of the study; and between(b) three different time points within the same group.

Results

Food and nutrient intakes

The mean food intake of children for all the three groups is presented in Table 1, and the percentage of RDA met is presented in Table 2. Food and nutrient intake of the children was recorded and calculated without including the daily interventions of massive Vitamin A dose/ RPO supplements.

Table 2. Mean food intake of children as percentage of RDA met.

Groups	Cereal	Pulses	GLV	OV	Milk	Fat
Massive dose (n=12)	102	77	55	88	49	49
4g RPO (n=12)	92	74	60	88	56	53
8g RPO (n=12)	90	67	54	94	49	61

GLV = green leafy vegetables; OV = other vegetables.

Almost no children met the recommended dietary allowances except children with massive dose group who were able to meet their cereal requirement. For cereals and vegetables the remaining two groups fell short by only 10%. No significant differences in food intake were observed between the three groups.

Anthropometric status

Mean nutrient intakes are presented in Table 3. Vitamin A and iron intakes were found to be less in the 8g RPO group compared to other groups ($P<0.05$). None of the groups met the requirement of any of these nutrients, especially with respect to iron (Table 4). Energy and vitamin A were met at around 63-70%, and protein at 70-80% in the first two groups. The anthropometric data for all children (Table 5) by grade of nutrition (Table 6) are shown.

Table 3. Mean nutrient intake of children.

Groups	Energy (Kcal)	Protein (g)	Vitamin A (µg/d)	Iron (mg)
Massive dose (n=12)	1365±399	34±9	1743±345	14±8
4g RPO (n=12)	1280±353	34±9	1719±296	12+9
8g RPO (n=12)	1283±339	30±9	1521**±399	7**±3
RDA	1950	41	2400	26

Values are mean ± SD; ** denotes significant differences between groups ($P<0.05$).

Table 1. Mean food intake of children (g)

Groups	Cereals	Pulses	GLV*	OV#	Milk	Fat
Massive dose (n=12)	254.3±80.7	54.0±14.8	41.0±14.1	44.2±7.6	122.1±54.2	14.7±5.5
4g RPO (n=12)	230.4±67.1	51.8±18.9	45.0±9.5	43.8±8.3	139.9±47.1	15.8±6.2
8g RPO (n=12)	224.9±68.7	47.0±17.9	40.5±12.5	46.9±11.9	122.0±59.1	18.3±6.1
RDA	250	70	75	50	250	30

Values are mean ± SD; * denotes green leafy vegetables. # denotes other vegetables.

Table 4. Mean nutrient intake of children as percentage of RDA met.

Groups	Energy	Protein	Vitamin A	Iron
Massive dose (n=12)	70	84	73	55
4g RPO (n=12)	66	82	72	45
8g RPO (n=12)	66	73	63	25**

**denotes significant differences (P<0.05) between rows.

Table 5. Mean anthropometric measurements of children.

Groups (n=12)	Height (cm)	Weight (kg)	Mid-arm circum (cm)
Massive dose	116± 7.1	19± 2.5	14± 1.3
4g RPO	118± 6.8	20± 1.7	13± 1.1
8g RPO	116± 8.2	19± 2.1	14± 1.2

Values are mean ± SD.

Table 6. Percentage distribution of children according to grades of nutrition.

Grade	Massive dose (n=12)	4g RPO (n=12)	8g RPO (n=12)	Total
Normal	17±2	25±3	17±2	19±7
Wasted	25±3	33±4	33±4	31±11
Stunted	41±5	33±4	50±6	42±15
Stunted + wasted	17±2	8±1	0	8±3

Values are mean ± SD.

Clinical assessment

Table 7 shows the occurrence of clinical signs in the subjects at baseline (I) and at the end (F) of the study period. No changes in clinical sign frequency were observed following supplementation.

Table 7. Percent distribution of children according to clinical signs.

Clinical symptoms	Number of children: Massive dose (n=12) I	F	4g RPO (n=12) I	F	8g RPO (n=12) I	F
Conjunctival Xerosis	75 (9)	75 (9)	75 (9)	75 (9)	58** (7)	58** (7)
Bitot's spots	25 (3)	25 (3)	25 (3)	25 (3)	42** (5)	42** (5)

I = Initial; F = Final; ** denotes significant differences between groups.

Serum retinol levels

Mean serum Vitamin A levels are shown in Table 8. Significant differences were observed between initial and intermediate Vitamin A levels, and also between initial and final levels in the massive dose group. Within the 4g RPO group, initial and final levels differed significantly at the 1% level from intermediate values. In the 8g RPO group, values at all three periods differed significantly from each other at the 1% level.

Initial levels in all groups were the same. In the final period, the 4g RPO group had the lowest levels (P<0.01). The percentage of subjects with serum retinol levels <0.7 µmol/l is indicated in Table 9. Initially, almost all children (80-92%) had serum retinol levels <0.7 µmol/l. After 15 days of supplementation, none of the children fell into this category. Finally, 3 months after cessation of supplementation, only four subjects in the 4g RPO group (33%) had levels <0.7 µmol/l.

Table 8. Mean serum retinol levels of children of the three groups.

Groups	Serum retinol level (µmol/l) Initial	Intermediate	Final
Massive dose	0.56±0.11**	1.07±0.25	0.90±0.23
4g RPO	0.53±0.12**	1.05±0.27	0.67±0.10**@
8g RPO	0.60±0.13**	1.79±0.70@#	0.97±0.62

Values are mean ± S.D. Intermediate - after 15 days of supplementation ; Final - after 3 months of supplementation. **denotes significant differences between columns; @ denotes significant differences between rows; # denotes significantly different from all groups (P<0.01).

Table 9. Percentage distribution of children with serum retinol levels < 0.7 µmol/l in different groups

Groups (n=12)	Serum retinol level (µmol/l) Initial	Intermediate	Final
Massive dose	92	0	0
4g RPO	92	0	57(33)
8g RPO	83	0	0

Discussion

Food and nutrient intakes

When the mean food intake was compared with RDA, none of the children met the standards, except the children of the massive dose group who were able to meet their cereal requirement (Table 1). Green leafy vegetables were consumed in a very small quantity because of parental ignorance of their nutritional value. Fruits like papaya and vegetables like yellow pumpkin and carrot were not consumed at all. About 50% of children consumed egg and meat only once in a month. So none of the children were found to meet the vitamin A requirement.

In a similar study carried out in preschool children of urban slums of Hyderabad, it was reported that the daily intake of vitamin A was far below the requirement, ranging from 60 to 100µg whereas the recommended level is 300µg[8]. Proteins and fats are essential for the absorption and utilisation of vitamin A, but the intakes of protein and fat were also low and none of the children met the requirements. These will be reasons for vitamin A malnutrition in these Orissa children.

Anthropometric status

Nearly 80% of the children had grades of nutrition which reflect an inadequate and poor quality of food intake over a long period of time. This poor dietary intake will in turn predispose to vitamin A deficiency. Again, inadequate and poor quality food intake leads to malnutrition and infections which further contribute to vitamin A deficiency. Corneal xerophthalmia often precedes an episode of infection[9], constituting a vicious cycle.

Clinical assessment

In the final round of clinical assessment, the same number of cases as that of the initial round was found to have conjunctival xerosis or Bitot's spots. Though in most of the cases the severity of conjunctival xerosis and Bitot's spots was reduced with 15 days RPO supplementation, they were not completely reversed. The reason may be the short period of supplementation. Sivakumar[10] states that clinical diagnosis, though commonly used, has its own limitations with subjective error and non-specificity. Clinical methods used in conjunction with biochemical analysis or any other improved functional methods like Relative dose response test and conjunctival impression cytology are more accurate. For practical reasons these methods were not used. However, it was encouraging that there was no case where vitamin A deficiency increased after 15 days of supplementation clinically or biochemically.

Biochemical assessment

Though serum retinol levels of these children were initially much lower than normal (≤0.7 μmol/l), after 15 days of supplementation with massive vitamin A dose and RPO at two different levels (4g and 8g), their serum retinol levels increased significantly. After 3 months of termination of supplementation, their retinol levels were still significantly higher than the initial level, but considerably below the concentration seen after 15 days supplementation.

Among both groups of RPO supplementation, the final serum retinol levels of 8g RPO group were higher compared to 4g RPO group. The 8g RPO group was supplemented with 50,000 IU of vitamin A from RPO (120g) for the entire 15 days of supplementation. Obviously, this group was able to maintain the serum retinol level beyond three months even after cessation of supplementation and was able to give protective effect as that of massive dose group. In the massive dose and 8g RPO groups, liver stores seemed to have built up adequately to meet requirements for the entire non-supplemented period, in spite of ongoing diets low in β-carotene or vitamin A. But in the 0-4 group which provided only 25,000 IU of vitamin A for the entire 15 days of supplementation, protection afforded was not equal to that achieved for the massive dose group. However, even in this group, a marginal increase in serum retinol was seen at the end of the three month period of non-supplementation, compared to baseline.

Relationship between anthropometry and retinol levels

When serum retinol levels were compared in relation to grades of malnutrition (Table 10), almost the same trend was observed among anthropometrically acceptable as for malnourished children, with supplementation. Initially (VAI), in the massive dose group, anthropometrically "normal" children had significantly higher ($P<0.01$) serum Vitamin A levels in comparison with all other grades of malnutrition. But in the other two treatment groups, all children had similar levels, irrespective of anthropometry.

The percentage increase after supplementation (VAIM) was >100% in normal and wasted children (Table 10), and around 75% in stunted, and wasted + stunted children, in the massive dose group. Final levels (VAF) were also higher in the "normal" children of this group.

In the 4g RPO group, normal and stunted children had a higher percent increase from initial (VAI), after 15 days supplementation (VAIM) and wasted children had only an 8% increase at the end of the study period (VAF).

In the 8g RPO group, a 237% increase was found in "normal" children whereas in wasted and stunted children it was 187% and 153% respectively, at the intermediate point (VAIM). Final (VAF) levels were significantly higher in "normal" children.

In spite of these differences, in all grades of nutritional status, supplementation improved Vitamin A status.

A study conducted by Pee *et al*[11], indicates that β-carotene from dark green leafy vegetables was poorly absorbed in comparison with enriched wafers in lactating women with low haemoglobin status. No improvement in Vitamin A status was observed in women fed vegetables or in control wafer groups in comparison with enriched wafer fed groups. Hume and Krebs[12] reckon that bioavailability of β-carotene from vegetables and carrots is only a third of that of β-carotene in oil. Since RPO contains β-carotene naturally in the oil itself, it also may be more bioavailable, as suggested in the present study.

Continuous consumption of carotene rich sources like RPO may not be necessary all through the year, as intermittent bouts of RPO may be sufficient to maintain serum levels. Longitudinal studies using larger sample sizes would be worthwhile. Given the limitations in long term use of massive vitamin A doses, twice a year[13], periodic intakes of RPO twice or thrice a year may prove to be equally effective in maintaining adequate nutritional status in vulnerable children, especially if it can be a food-based approach.

Summary

RPO was found to be equally effective in maintaining serum retinol levels as megadose vitamin A in those prone to vitamin A deficiency. In planning supplementary feeding programmes, rather than regular daily feeding, periodic feeding of RPO at regular three monthly intervals may be successful in maintaining normal childhood vitamin A nutriture.

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Table 10. Mean serum vitamin A (μmol/l) levels amongst clinically vitamin A deficient children in relation to nutritional status, indexed by anthropometry.

Nutritional status	Massive dose			4g RPO			8g RPO		
by anthropometry	VAI	VAIM	VAF	VAI	VAIM	VAF	VAI	VAIM	VAF
Normal	0.71*	1.49	1.31	0.59	1.46*	0.75	0.57*	1.92	1.42
% Increase		(109)	(85)		(147)	(27)		(237)	(149)
Wasted	0.44*	0.99	0.74	0.53	0.86*	0.57	0.56*	1.61	0.86*
% Increase		(125)	(68)		(62)	(8)		(187)	(54)
Stunted	0.59*	1.02	0.83	0.50	1.02*	0.69	0.64*	1.62	0.88*
% Increase		(73)	(41)		(104)	(38)		(153)	(38)
Wasted + stunted	0.50*	0.89	0.79	0.45*	0.78	0.68	-	-	-
% Increase		(78)	(58)		(73)	(51)			

* denotes significantly different treatment responses ($P<0.01$), within the same group.
VAI = vitamin A initial; VAIM = vitamin A after 15 days of supplementation; VAF = vitamin A finally, 3 months after supplementation

The protective effect of red palm oil in comparison with massive vitamin A dose in combating vitamin A deficiency

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比較紅棕櫚油 (RPO) 與大劑量維生素 A 對抗維生素 A 缺乏病時的保護作用

摘要

長期以來兒童的維生素 A 缺乏是一種嚴重的世界公共危害。兒童發病率高的主要原因是膳食中缺乏維生素 A。富含胡蘿白素的紅棕櫚油的補充可用以對抗維生素 A 缺乏病。該研究比較了紅棕櫚油與大劑量維生素 A 在對抗維生素 A 缺乏病時的保護作用。作者選用了 36 位維生素 A 缺乏的學齡兒童進行 3 個月試驗。12 位兒童每日服用大量維生素 A（50,000 國際單位），另 12 位兒童每日服用含有相當於 25,000 國際單位維生素 A 的 β–胡蘿白素的 RPO 4 克，餘下 12 位兒童每日服用含有相當於 50,000 國際單位維生素 A 的 β–胡蘿白素的 RPO 8 克。在試驗開始，補充 15 日後和 3 個月後結束時分別測定血清維生素 A 水平。結果顯示，補充 15 天後血清維生素 A 濃度最高，雖然補充 3 個月後下降，但三組兒童仍顯著高於試驗開始時的血清維生素 A 水平。在補充 RPO 兩組兒童中，補充 8 克 RPO 組與補充大劑量維生素 A 組一樣有效，停止補充 3 個月後仍有保護作用。

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