Community and International Nutrition

Supplementing Lactating Women with Puréed Papaya and Grated Carrots Improved Vitamin A Status in a Placebo-Controlled Trial[1]

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ABSTRACT Doubts have been raised about the effectiveness of carotene-containing foods in improving the vitamin A status of populations at risk. We investigated the effect of papaya and carrots on the vitamin A status of lactating women with 2- to 12-mo-old infants in Zimbabwe. The women were randomly assigned to three supplementation groups and a placebo group, and received 6 mg of β-carotene capsules, 650 g puréed papaya, 100 g grated carrots or a placebo, daily for 60 d. All groups were given a meal containing 10 g of vegetable oil daily. Serum retinol, relative dose response, serum ferritin, hemoglobin and C-reactive protein were measured before and after the supplementation period. Mean serum retinol increased significantly after supplementation in the β-carotene group ($P < 0.001$), the papaya group ($P < 0.001$) and the carrot group ($P < 0.001$), but not in the placebo group ($P > 0.05$). The relative dose response decreased significantly ($P < 0.05$) in the β-carotene and papaya groups, but not in the carrot or placebo groups ($P > 0.05$). There was an increase in mean serum ferritin in all groups but the increase did not differ among groups. The hemoglobin increases in the β-carotene and papaya groups were greater than that in the placebo group. We conclude that puréed papaya and grated carrots can improve the vitamin A and iron nutriture of lactating women. These findings reinforce the importance of plant food–based approaches in the control of vitamin A deficiency in low income countries. J. Nutr. 131: 1497–1502, 2001.

KEY WORDS: • *vitamin A* • *β-carotene* • *supplementation* • *lactating women* • *Zimbabwe*

Traditionally, public health strategies to improve vitamin A status have focused on increased consumption of carotene-rich fruits and vegetables as the most appropriate and sustainable solution for populations in low income countries (1). The plan of action of the International Conference of Nutrition in 1992 prioritizes diet-based approaches for low income countries. However, recent reports have raised doubts about the effectiveness of this approach, particularly the increased consumption of green leafy vegetables (2–4). Several reasons may have contributed to this lack of improvement of the vitamin A status with plant food–based interventions, i.e., the relatively satisfactory vitamin A status at baseline, the dose of β-carotene given and the bioavailability of the plant food source.

It has also been suggested that vitamin A supplementation increases the packed-cell volume and hemoglobin (Hb)[3] concentration in subjects with low vitamin A status and low Hb (5). However, data regarding lactating women in this respect are scarce. No such information is available in Zimbabwe.

The bioavailability of carotenoids is influenced by a number of factors such as the carotenoid species ingested, the amount of carotenoid in the diet, the matrix of the food source, the presence of absorption enhancers or inhibitors, other host-related factors and interactions among these factors (6). The uptake and absorption of carotenoids may be inhibited by factors such as the particle size and the location of the carotene in the plant (7). Processing the food or reducing particle size makes the carotenoids more available. The bioavailability of β-carotene from dark green leafy vegetables is lower than that from fruits (8) and both are lower than that from pure β-carotene (2,8,9). Furthermore, cooked or processed vegetables have been shown to have more bioavailable β-carotene than raw vegetables (10).

Although most vitamin A research has focused on children because of this vitamin's demonstrated role in child health, growth and survival, inadequate attention has been paid to the health and nutrition of women in low income countries in which vitamin A deficiency may be prevalent. As awareness of the importance of vitamin A deficiency in women increases (11), it is important to focus on lactating women whose vitamin A requirements exceed even those in pregnancy. If the vitamin A intake is not sufficient to replace the amount transferred to the infant through breast milk, the maternal vitamin stores may become depleted (12). It is estimated that lactating women in low income countries have an average daily intake of vitamin A that is less than half that of lactating women in high income countries (13). In a recent report on

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[3] Abbreviations used: CRP, C-reactive protein; Hb, hemoglobin; RDR, relative dose response.

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the vitamin A status of lactating women in the arid area of Makhaza in Zimbabwe, we found that 40% of the women were deficient in vitamin A from their serum retinol levels and 76% were deficient based on relative dose response (RDR) (14). These findings stress the urgency of the need to redress the vitamin A situation in this area. The purpose of this study was to examine the effect of supplementing lactating women with grated carrots, puréed papaya and β-carotene in oil capsules on their vitamin A status and iron status.

SUBJECTS AND METHODS

Study design. The study was a 60-d placebo-controlled supplementation trial undertaken to examine changes in the vitamin A and iron status of lactating women after they had been randomly assigned to one of the following treatment groups: group 1 received 6 mg β-carotene in oil capsules; group 2 received 650 g puréed papaya; group 3 received 100 g grated carrots; and group 4 received a placebo, which was a teaspoonful of pink-colored water.

Supplements. The daily papaya and carrot portions were measured to approximate 6 mg of β-carotene, using the conversion factor of 6 and the South African Food Composition Tables (15). The carotinoid content of carrots was 1100 μg; that of papaya was 175 μg/100 g edible portion. The β-carotene in oil was supplied in 6-mg brown capsules from Lancaster Industries (Cape Town, South Africa); the placebo was pink-colored water, and each woman assigned to the placebo group was given one teaspoonful daily. Papayas were washed, cut in half and all seeds were removed; the inner pulp was then scooped out into a bowl and puréed, and 650 g was weighed out for each woman in the papaya group. Carrots were washed and grated and 100 g was weighed out for each woman in the carrot group. The supplements were provided daily between 1000 and 1200 h from a central point within each village conveniently selected by the women themselves. The women were trained in the daily preparation and portioning of the supplements and were supervised by nurses from the local health center and by the principal investigator (T.N.). A meal containing at least 10 g of vegetable oil and no vitamin A was supplied daily to all of the women in the study throughout the 60-d supplementation period.

The puréed papaya and grated carrots supplements were both well accepted by the women. The large glass of ripe puréed papaya was a welcome refreshment on the hot September and October summer days during which the study was conducted. The supplements were given before the corn/bean meal was given when the women were still hungry and thirsty; no wastage was recorded. Toward the end of the study, the carrot group in one of the villages wanted to chew whole carrots, but when the purpose of grating and the need for uniformity was explained to them, they complied with no problem.

Subjects. The study population consisted of lactating women with infants aged between 2 and 12 mo and living in the twelve villages comprising the Makhaza area of Tsholotsho district in Zimbabwe. After a house-to-house registration of such women, 211 were found eligible. Of these, two declined to participate and two others were excluded due to illness. One of the latter two required medical attention and was referred to the local clinic; the other had Bitot's spots and was treated and excluded from the study.

The sample size required for each of the four treatment groups was calculated to be 46 at 95% confidence interval, providing 90% power to be able to detect a difference of 0.4 μmol/L with a SD of 0.2 μmol/L in serum retinol between the supplementation and placebo groups. This sample size was rounded up to 50. After the above 4 exclusions, 207 eligible women gave written consent and were enrolled. The women were then randomly assigned to the four supplementation groups. The four groups were labeled A, B, C and D before registration; as the women were registered, they were systematically assigned to each group in the order in which they arrived. After registration, when each woman had a group, the groups were then assigned supplements. Group A was assigned β-carotene, referred to as medicine A; group B was assigned placebo (pink colored water, designed to match the β-carotene capsule, and referred to as medicine B); group C was assigned papaya, referred to as food A; and group D was assigned carrots, referred to as food B. **Figure 1** shows how the sample

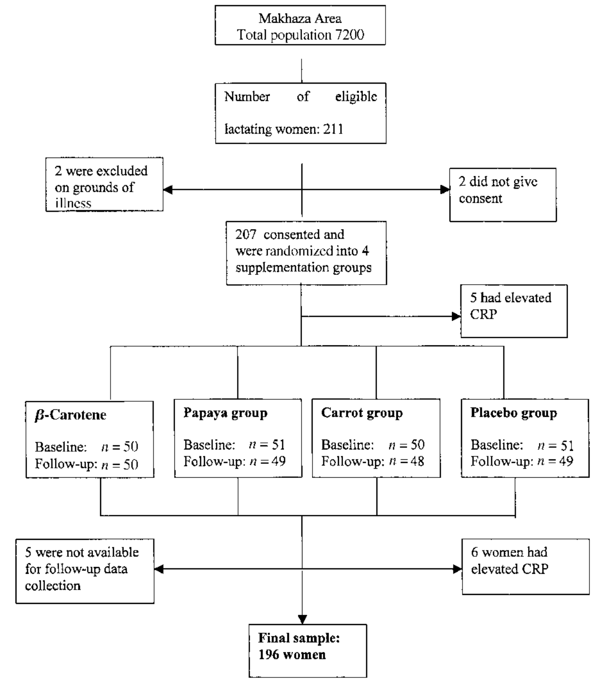

FIGURE 1 Allocation of the study population into β-carotene, papaya, carrot and placebo groups. CRP, C-reactive protein, is an indicator of acute infections.

of 207 women was selected and allocated to the four treatment groups. The study was approved by the Ethical Committee of the Medical Research Council of Zimbabwe and the Ministry of Health and Child Welfare in Zimbabwe.

Anthropometric measurements. The weight of the mothers was measured to the nearest 100 g, using SECA ALPHA 770 electronic adult scales. The scales were standardized and checked each day against a known weight. Height was measured to the nearest 0.5 cm using a mounted tape measure. Body mass index was calculated as weight (kg)/height (m)^2. Mid-upper arm circumference was measured to the nearest 0.1 cm with a tape measure, on the left arm, with the arm in an extended position, mid-way between the elbow and the acromion. Height was measured only at baseline, and the other anthropometric measurements were taken before and after supplementation.

Blood sample collection. Blood samples were collected before and after supplementation. Venous blood (~5 mL) was taken from the cubital vein in each mother. A senior qualified medical laboratory technologist from Uppsala University together with two nurses collected the blood under the supervision of a physician. The blood samples were taken from nonfasting subjects between 0800 and 1000 h, placed on ice and protected from light by wrapping them in aluminum foil. All blood samples were transported within 8 h to the laboratory and centrifuged (300 × g, 5 min); the serum was separated, frozen and kept at −20°C for the 7 d during which fieldwork was done. The serum samples were subsequently stored at −70°C at the University of Zimbabwe for 2 mo until transported on dry ice to Sweden and stored at −70°C until analyzed 6–8 mo later.

A RDR test was conducted on every fifth woman selected systematically from the 207 subjects. A subsample of 43 women was selected, and a dose of 1.75 μmol of retinol palmitate in an oil suspension was administered to them orally immediately after the baseline blood sample had been taken. A follow-up blood sample was taken 5 h later. While waiting for the follow-up blood sample, the women were provided with a vitamin A–free snack of plain white bread and a cup of black tea with sugar. The RDR was calculated as a percentage: $RDR = (A_5 - A_0)/A_5 \cdot 100$ where A_5 is the serum retinol concentration 5 h after the dose and A_0 is the concentration immediately before the dose was given (16).

Analytical procedures. HPLC was used to determine the serum retinol levels. Serum proteins were denatured by addition of aceto-

TABLE 1

Baseline characteristics of the lactating women in the three supplemented groups compared with the placebo group[1,2]

	β-carotene	Papaya	Carrots	Placebo	Total
n	50	51	50	51	202
Age of infant, *mo*	6 ± 3	7 ± 3	7 ± 3	7 ± 3	7 ± 3
Mother's age, *y*	28 ± 7	26 ± 8	27 ± 7	27 ± 7	27 ± 7
Parity, *n*	4 ± 2	3 ± 2	3 ± 2	3 ± 2	3 ± 2
BMI, *kg/m*^{2}	22 ± 3	22 ± 2	22 ± 3	22 ± 2	22 ± 3
MUAC, *cm*	27 ± 3	27 ± 3	27 ± 3	27 ± 3	27 ± 3
Serum retinol, *μmol/L*	0.9 ± 0.4	0.9 ± 0.4	0.8 ± 0.3	0.9 ± 0.4	0.9 ± 0.4
RDR, %	44 ± 22	35 ± 19	36 ± 30	48 ± 18	41 ± 23 (*n* = 43)
Hemoglobin, *g/L*	120 ± 20	120 ± 10	130 ± 10	130 ± 20	120 ± 20
Serum ferritin, *μg/L*	25 ± 15	24 ± 10	24 ± 14	24 ± 13	25 ± 13 (*n* = 161)

1 Values are means ± SD.
2 BMI, body mass index; MUAC, mid-upper arm circumference; RDR, relative dose response.

nitrile, and α-tocopherol acetate was added as the internal standard for retinol. Retinol was subsequently extracted into an organic matrix consisting of ethyl acetate/butanol (1:1), and eluted by reversed-phase chromatography from a C18 column with an isocratic mobile phase of methanol/water (95:5). UV detection at 325 nm was used to identify analytes. The method gave same-day and between-day CV of 3.5 and 4.8, respectively.

Serum ferritin was assayed with a double monoclonal antibody technique with a CV of 4.2% at 135 mg/L and 7.8% at 14.9 μg/L. Serum ferritin assays were performed on a subsample of 163 women; the other 39 samples were insufficient for analysis. C-reactive protein (CRP) was assayed by a turbidometric method. The measuring interval was 0–175 μg/L, with a reference interval of <10 μg/L and a CV of 10% at 20 μg/L. Levels ≥10 μg/L were considered an indication of ongoing acute infection and were dealt with separately in the data analysis.

Statistical analysis. For analysis of data, the SPSS/PC statistical package for the social sciences, version 10, was used. Means, medians and SD were calculated for all normally distributed data; medians and the 25th–75th percentiles were calculated for data that were not normally distributed. Independent sample *t* test and ANOVA were used to test differences between groups. The paired *t* test was used to test differences between baseline and endpoint values, and Dunnett's test was used to compare supplementation groups with the control groups. Dunnett's test is a pairwise multiple comparison *t* test that compares a set of treatments against a single control mean (17). The differences between variables were considered significant at $P < 0.05$.

RESULTS

Baseline data were collected from 207 lactating women. At baseline, 5 (2%) women had elevated CRP (>10 μg/L), with a mean CRP of 50 ± 25 mg/L and a mean serum retinol of 0.3 ± 0.2 μmol/L. At endpoint, these 5 women and one additional woman had elevated CRP, with a mean CRP of 30 ± 4 μg/L and mean serum retinol of 0.4 ± 0.1 μmol/L. The serum retinol concentrations in these 6 women were all <0.70 μmol/L. Women with elevated CRP were excluded from the analysis to adjust for subclinical infections. Baseline characteristics for the supplementation and placebo groups are given in **Table 1**. At endpoint, 5 women were away and not available for any tests. Endpoint data were thus collected from 202 supplemented women 5 d after the end of the supplementation period. Women were requested not to change their diet during this period.

Effect of supplementation on vitamin A status. The effects of the different supplementations on serum retinol concentrations are shown in **Figure 2** and **Table 2**. Serum retinol increased in the β-carotene, papaya and carrot groups ($P < 0.001$), but there was no increase in the placebo group. A clear shift of the distribution to higher values as a result of the intervention was evident. A subsample of 43 women was tested for liver stores, using the RDR method. In the β-carotene and papaya groups, the mean RDR decreased significantly from baseline to endpoint compared with the placebo group ($P < 0.05$). The carrot and the placebo groups showed no improvement in vitamin A status. The proportion of women with low liver stores (RDR >20%) decreased from 78% at baseline to 33% at endpoint in the β-carotene group, and from 58 to 33% in the papaya group. The mean differences in serum retinol in the three supplemented groups compared with the placebo group were all significant ($P < 0.05$). The mean differences in RDR compared with the placebo group were significant for the β-carotene and papaya groups, but not for the carrot group ($P < 0.05$).

Effect of supplementation on anemia and iron status. The effect of supplementation on the Hb levels is shown in **Figure 3** and **Tables 2** and **3**. Hemoglobin levels increased in all groups including the placebo group, but the mean increase was greater in the β-carotene and papaya groups than in the

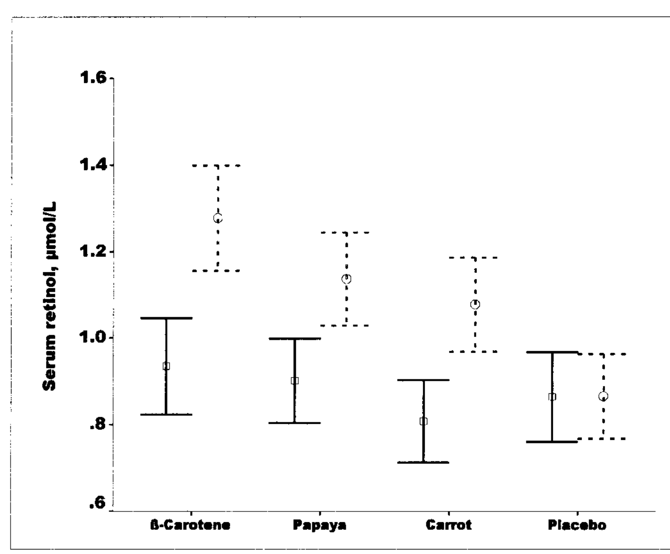

FIGURE 2 Significant changes in mean serum retinol and 95% confidence interval in lactating women supplemented with β-carotene, papaya and carrot compared with the placebo group after a 60-d supplementation trial. Squares (□) are baseline means and 95% confidence interval and circles (○) are endpoint means and 95% confidence interval. Endpoint values are after a 60-d supplementation trial.

TABLE 2

Effects of β-carotene, papaya and carrot supplementation of lactating women on serum retinol, relative dose response, ferritin and hemoglobin[1]

	β-Carotene (n = 50)		Papaya (n = 49)		Carrot (n = 48)		Placebo (n = 49)	
	Before	After	Before	After	Before	After	Before	After
Serum retinol, μmol/L								
Mean ± SD	0.9 ± 0.4	1.3 ± 0.4	0.9 ± 0.3	1.1 ± 0.4	0.8 ± 0.3	1.1 ± 0.4	0.9 ± 0.4	0.9 ± 0.3
Mean paired change	0.3 ± 0.4***		0.2 ± 0.3***		0.3 ± 0.3***		0.0 ± 0.2	
<0.70 μmol/L,[2] %	38	10	35	10	44	17	49	37
Relative dose response (RDR),[3] %								
Mean ± SD	44 ± 22	21 ± 21	35 ± 19	15 ± 22	36 ± 30	25 ± 20	49 ± 18	42 ± 21
Mean paired change	−23 ± 33*		−20 ± 29*		−10 ± 31		−7 ± 18	
>20%, %	78	33	58	33	82	64	91	73
Serum ferritin, μg/L								
Mean ± SD	24.9 ± 15	31.2 ± 16	24.0 ± 10	30.6 ± 15	24.1 ± 14	30.0 ± 15	23.3 ± 13	28.9 ± 19
Mean paired change	6.6 ± 10		6.0 ± 15		6.7 ± 12		6.5 ± 20	
<12 μg/L, %	10	7	11	3	12	5	18	16
Hemoglobin, g/L								
Mean ± SD	12.4 ± 2	13.6 ± 1	12.2 ± 1	13.3 ± 1	12.4 ± 1	13.1 ± 1	12.6 ± 2	13.1 ± 2
Mean paired change	1.2 ± 2***		1.1 ± 2***		0.7 ± 1*		0.4 ± 1	
<120 g/L, %	44	6	43	16	33	21	35	29

1 Values are means ± SD before and after 60 d of supplementation.
2 Mean changes between baseline and follow-up are significant at: * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$.
3 For RDR, n = 9, 12, 11 and 11 for the β-carotene, papaya, carrot and placebo groups, respectively.

placebo group. Of 15 women with anemia at baseline in the β-carotene group, only 2 (13%) remained anemic after supplementation; in the papaya group, 5 of 13 remained anemic, whereas only 3 and 2 improved in the carrot and placebo groups, respectively. The mean differences in the three supplemented groups compared with the mean difference in the placebo group were not significant ($P > 0.05$).

The effects of β-carotene supplementation on serum ferritin are shown in Tables 2 and 3. However, there was an increase in the median serum ferritin concentration (25th–75th percentile) from baseline to endpoint in the three supplemented groups but not in the placebo group. The median (25th–75th percentile) serum ferritin level in all women with normal CRP (n = 158) increased from 22 (16–29) μg/L at baseline to 28 (20–38) μg/L at endpoint ($P < 0.001$). The proportion of women with iron deficiency (serum ferritin

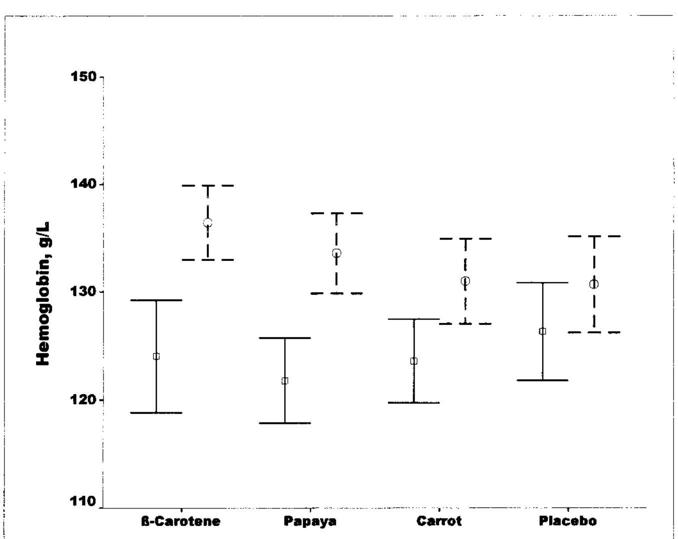

FIGURE 3 Significant changes in mean hemoglobin and 95% confidence interval in lactating women supplemented with β-carotene and papaya compared with the placebo group after a 60-d supplementation trial. Squares (□) are baseline means and 95% confidence interval and circles (○) are endpoint means and 95% confidence interval. Endpoint values are after a 60-d supplementation trial.

TABLE 3

Lactating women with anemia, iron deficiency and iron-deficiency anemia in the β-carotene, papaya and carrot groups compared with placebo at baseline and at endpoint after a 60-d supplementation trial[1]

	n	Normal[2]	Anemia[3]	Iron deficiency[4]	Iron-deficiency anemia[5]
			n		
β-Carotene					
Baseline	42	23	15	2	2
Endpoint	41	36	2	3	0*
Papaya					
Baseline	36	19	13	2	2
Endpoint	33	27	5	1	0*
Carrot					
Baseline	41	24	14	2	1
Endpoint	38	27	9	2	0*
Placebo					
Baseline	39	22	10	5	2
Endpoint	38	22	8	4	4

1 All women with iron deficiency anemia in the supplementation groups were no longer deficient at end point.
2 Normal, hemoglobin ≧120 g/L and serum ferritin ≧12 μg/L.
3 Anemia, hemoglobin <120 g/L.
4 Iron deficiency, serum ferritin <12 μg/L.
5 Iron deficiency anemia, hemoglobin <120 g/L and serum ferritin <12 μg/L.
* $P < 0.05$

<12μg/L) decreased from 13% at baseline to 8% ($n = 158$) at endpoint. All women in the three supplemented groups who had iron-deficiency anemia (serum ferritin <12 μg/L and Hb < 120 g/L) at baseline improved to a normal status after supplementation, but the two women who had iron-deficiency anemia in the placebo group did not improve and two more in that group became deficient.

Difference in effect among supplements. The effect of papaya appeared to be approximately equivalent to that of β-carotene capsules. There were no significant differences in their effectiveness for any variable measured. Furthermore, there were no significant differences between the carrot and the papaya groups. However, the effect on serum retinol was significantly poorer in the carrot group than in the groups that received β-carotene capsules.

DISCUSSION

In this study, we investigated the effect of β-carotene supplementation on the vitamin A and iron nutriture of lactating women. This was a placebo-controlled population-based study with nearly complete coverage of lactating women with infants aged 2–12 mo living in the semiarid area of Makhaza in Zimbabwe. Five women (2%) showed elevated CRP values at baseline and six women (3%) at endpoint. These women were excluded from the analysis. They had the lowest serum retinol levels in the series, underscoring the importance of including indicators of subclinical infections to assess the effect of intervention.

The supplements effectively increased the mean serum retinol concentrations in all treatment groups, but not in the placebo group. The mean decrease in the RDR in the carrot group was not significant ($P > 0.05$), nor was it different from that in the placebo group. Not only were the mean values of the different indicators improved in the treatment groups, but the proportion of women below the WHO cut-off points for all indicators was also reduced.

Of great interest regarding the effect of diet-based approaches is the type of carotene-containing food used, as well as the type of meal eaten with it. In our study, we chose papaya and carrots because both are high in carotene, they can be grown by and are readily acceptable to the rural population of Zimbabwe, and they represent a fruit and a root vegetable, respectively. Our study showed that grated carrots and puréed papaya do improve the vitamin A and iron status of lactating women. It is likely that the partial processing, i.e., the grating of the carrots and the puréeing of the papaya, enhanced the bioavailability of the carotenes in these two supplements. Both supplements used in our study were processed by methods familiar and acceptable to the population studied. Recent studies have shown that processing of carrots might improve the bioavailability of their β-carotene. Brown et al. (8) provided 29 mg of β-carotene in a single meal of cooked carrots and reported a bioavailability of 21%; Huang et al. (18) provided 12 mg of β-carotene in stir-fried shredded carrots and reported a bioavailability of 33%; and Torronen et al. (19) provided 12 mg of β-carotene in carrot juice daily for 6 wk and reported a bioavailability of 45%. In all of these studies, carrots in different forms were used as supplements and their bioavailability seemed to increase with processing, with carrot juice showing the highest bioavailability.

All subjects in our supplementation trial, including those in the placebo group, were given a daily meal consisting of corn, beans and vegetable oil after taking the supplement. The meal was given to minimize the effects of a low fat diet. Fat added to dark green leafy vegetables has been shown to enhance serum retinol levels after children were supplemented for 12 wk (20). Shiau et al. (21) reported that 35–71% labeled β-carotene was recovered as fecal losses when β-carotene was taken with a meal, compared with 83% without a meal. The dosage of β-carotene (6 mg) in our study approximated the recommended daily requirements for lactating women (22) and was given daily for 60 d. Repeated doses of β-carotene give less variable results than large single pharmacologic doses. Constantino et al. (23) and dePee et al. (24) found that the serum retinol response to β-carotene from fruits was four times that from green leafy vegetables. The enhanced response could be due to the fact that carotenoids in yellow-to-orange fruits are dissolved in oil droplets in chromoplasts and can be readily extracted during digestion. In green leafy vegetables, the β-carotene molecules are in pigment-protein complexes located in chloroplasts and are more difficult to extract. Carrots may be intermediate between these extremes in their carotenid bioavailability. The β-carotene in carrots exists in crystalline form, making it less bioavailable than that in orange and yellow fruits (25).

The addition of fat to the diet greatly improves the absorption of carotenoids (21,26,27). The meal that was given with the supplements in our study provided ~10 g of fat. The severely vitamin A–deficient women in our study showed a significantly greater response than women with a more normal vitamin A status. Similar observations were made in two other studies (28,29) in which the vitamin A status of malnourished children improved after consumption of green leafy vegetables.

Our findings show a reduction in the proportion of women with anemia in the supplementation groups but not in the placebo group. Similarly, all of the subjects with iron deficiency anemia in the supplemented groups had normalized levels at the endpoint but this was not the case in the placebo group. The general improvement in serum ferritin could have been a result of the meal given to all groups and might also be a reflection of the hemo-concentration women experience after pregnancy and delivery.

In conclusion, our study showed that β-carotene supplementation with yellow-to-orange fruits and vegetables improves the vitamin A and iron status of lactating women. In each of the supplemented groups, those who were severely malnourished showed significant improvements in all indicators, i.e., serum retinol, relative dose response, hemoglobin and serum ferritin.

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