Comparison of the effects of supplemental red palm oil and sunflower oil on maternal vitamin A status[1–3]

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ABSTRACT

Background: Conflicting results have been reported on the ability of dietary carotenoids to improve vitamin A status in lactating women. Red palm oil is one of the richest dietary sources of β-carotene.

Objective: We aimed to determine the efficacy of red palm oil in increasing retinol and provitamin A status in pregnant and lactating women.

Design: Ninety rural, pregnant Tanzanian women from 3 randomly selected villages were recruited during their third trimester to participate in 3 dietary intervention groups: a control group, who were encouraged to maintain the traditional practice of eating staples with dark-green leafy vegetables, and 2 study groups, who were given either sunflower or red palm oil for use in household food preparations. The intervention lasted 6 mo. Plasma samples were collected at the third trimester and 1 and 3 mo postpartum, and breast-milk samples were collected 1 and 3 mo postpartum.

Results: Supplementation with red palm oil, which is rich in provitamin A, increased α- and β-carotene concentrations significantly ($P < 0.001$) in both plasma and breast milk. Plasma retinol concentrations were similar in all dietary groups. Breast-milk retinol concentrations tended to decrease from 1 to 3 mo postpartum in the control group, but were maintained in both oil groups. The difference in change in breast-milk retinol concentration between the red palm oil group and the control group was significant ($P = 0.041$).

Conclusions: Consumption of red palm oil increases concentrations of α- and β-carotene in both breast milk and serum and maintains breast-milk retinol concentrations. Sunflower oil consumption seems to conserve breast-milk retinol similarly to consumption of red palm oil. Breast-milk retinol might be maintained through increased dietary intake of these vegetable oils and use of mild cooking preparation methods (such as the addition of oil at the end of cooking and avoidance of frying). *Am J Clin Nutr* 2001;74:501–9.

KEY WORDS Red palm oil, sunflower oil, vitamin A, α-carotene, β-carotene, pregnancy, lactation, breast milk, plasma, Tanzania, women

INTRODUCTION

For the past 3 decades, vitamin A deficiency has been recognized as a major public health problem in the developing world. Vitamin A status substantially affects mortality in both children and women through its effect on the incidence and severity of life-threatening infections (1, 2). Subclinical vitamin A deficiency has been observed in infants fed breast milk (3), suggesting that lactating women in developing countries may have insufficient vitamin A stores.

Various interventions have been proposed to improve the vitamin A status of women during pregnancy and lactation. These include low-dose supplementation during pregnancy (2), high-dose supplementation immediately postpartum (4, 5), fortification of food with vitamin A (6), and dietary interventions (7, 8). Regular supplementation is difficult to achieve in areas where women have limited access to health facilities. High-dose supplementation is possible during early lactation, but the teratogenic effects of high doses of vitamin A preclude its use during pregnancy. Furthermore, in one study the recommended single postpartum dose of 200 000 IU (209 μmol) retinol did not maintain maternal vitamin A status for more than a few months in Bangladeshi women (4). Food fortification may not be feasible, especially in rural areas where households produce their own food and no centralized food processing and distribution system exists. Such populations require improved diets that provide a safe intake of vitamin A throughout pregnancy and lactation. Whereas increases in serum β-carotene concentrations in children supplemented with β-carotene are well documented (9, 10), few studies have explored the relation between β-carotene intake in lactating women and the concentration of carotenoids in milk and serum (7, 11).

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Red palm oil may play a double role in improving vitamin A status by providing both provitamin A carotenoids and oil that promotes the absorption of vitamin A and provitamin A carotenoids. Red palm oil is the richest natural source of provitamin A carotene (12), and has been successfully used in supplementary feeding trials with children (13, 14). To examine the effect of dietary carotenoids independently of the beneficial effect of the oil consumed, our experimental design included a control group; a group fed sunflower oil, which lacks provitamin A; and a group fed red palm oil, which is rich in provitamin A.

SUBJECTS AND METHODS

Study area

The study was conducted in the Ilongero division of the Singida rural district in the drought-prone central part of Tanzania. Women were recruited from 3 different villages that were selected at random from a larger community of 20 villages. More than 90% of the population depends on agriculture, with livestock being the second most important household resource (15). Food security is reported to be inadequate for 74% of households in the region (16), and foods rich in vitamin A are relatively unavailable (17). The prevalences of serum retinol concentrations <0.7 and 0.35 μmol/L among children aged 1–6 y are 60% and 10%, respectively, indicating vitamin A deficiency of public health significance according to the World Health Organization (17). The population also has a low fat intake, with 35% of children relying on breast milk as their sole fat source; most mothers (74%) breast-feed their children for >18 mo (17).

Subjects

Ethical issues and considerations

The study was approved by the Research and Ethics Committee of the Tanzania Food and Nutrition Centre. The entire study was explained to all the women and it was made clear that the women were under no compulsion to enter or continue in the study. All community leaders, senior community health staff, and the district medical officer were informed of the objectives of the study and their support was obtained.

Exclusion criteria and group assignment

All women were invited to join the study when they attended antenatal clinics, provided they fulfilled the study criteria. Women were not enrolled if they were severely anemic (hemoglobin <70 g/L) or if they had severe clinical infections such as tuberculosis or HIV-related diseases as determined by verbal interviews. Similarly, those who had already started participating but could not use the dietary promotion techniques or refused to donate blood were excluded in the first month after enrollment. Other women were excluded 1 mo after enrollment when all subjects were reexamined by a midwife because they were found to have been recruited at >22 wk of gestation. After informed consent was obtained, 90 pregnant women in their third trimester were recruited.

The study was not blind because it was impossible to disguise the dietary treatments, and women were allocated to treatments based on practicality and to minimize crossover between groups. This was achieved by isolating the red palm oil and sunflower oil groups from each other by a distance of ≈3 km, with the control group situated in the village in between.

Study group characteristics

The dietary patterns, population structure, and ecology of the 3 study villages were comparable. The staple foods in the study community included sorghum, millet, maize, and sweet potatoes, and different legumes (beans or cowpeas) and a variety of green leafy vegetables constituted the common relish. On average, adults consumed 2 meals/d. Fish, meat, and eggs were rarely eaten. There were no significant differences between villages in a socioeconomic score derived from several variables; for example, all but one of the women were small-scale farmers, none of the villages had electricity, and all were at some distance from water supplies, especially in the dry season. The women were recruited simultaneously at the beginning of the dry season between June and August. There were no significant differences between villages in infant birth date (July to December) or in the amount of time before delivery that the women were given the oils ($\bar{x}$: 75 d; 95% CI: 68, 83 d).

Intervention

The 3 study groups were as follows: the control group (n = 30), who were encouraged to maintain their consumption of dark-green leafy vegetables and were given 4 kg rice/mo per family (a modest amount designed as an incentive rather than as a dietary supplement); the sunflower oil group (n = 30), who were advised to maintain their consumption of dark-green leafy vegetables and were given sunflower oil; and the red palm oil group (n = 30), who were advised to maintain their consumption of dark-green leafy vegetables and were given red palm oil. Oils were provided monthly throughout the third trimester and the first 3 mo postpartum. Adequate oil was supplied according to household size to ensure that the whole family would benefit from the treatment, and women were instructed to use 4 plastic tablespoons of oil for themselves (≈12 g) and to distribute the oil pro rata to other family members (3 tablespoons for adults and children aged >5 y, 2 tablespoons for children aged <5 y). Twelve grams was chosen as the quantity of oil to be consumed on the basis of the calculation that women would have consumed ≈209 μmol (≈200 000 IU) vitamin A by the end of the project (assuming that 1.8 μg *all-trans*-β-carotene, 3.6 μg *all-trans*-α-carotene, and 3.6 μg *cis*-β-carotene are equivalent to 1 IU vitamin A). Therefore, 116 μmol (111 480 IU) vitamin A would be derived from *all-trans*-β-carotene, 48 μmol (45 495 IU) vitamin A from *all-trans*-α-carotene, and 53 μmol (50 640 IU) vitamin A from *cis*-β-carotene. Cooking demonstrations were performed at the time of recruitment with use of either sunflower oil or red palm oil. Women were encouraged to incorporate the oil into local recipes and advised not to heat the oil for too long.

Researchers made a total of 6 visits (one every month) to the villages. Three visits were made before each woman delivered and 3 after delivery. For the red palm oil group, women assembled at the village dispensary. The sunflower oil group assembled at the village government offices because there was no dispensary in that village. The control group assembled at the primary school located in their village. At each location, separate rooms were used for interviewing (administering a questionnaire), drawing blood, expressing breast milk, and taking anthropometric measurements. Women received oil or rice at the end of these activities.

Compliance

Compliance was monitored by estimating how much oil should have been used (taking into account the number of household

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members) and then measuring the amount of oil remaining each month. Over- and underconsumption were observed either when the oil was exhausted after a short period of time (eg, from sharing with visitors) or when very little oil had been used (eg, because of the recommendations of a traditional healer). To closely supervise the recruited women, a research nurse and an agricultural division officer made random visits to each household to ensure that the oil was properly used. Volunteers who were using the oil inappropriately (eg, for frying) were closely monitored and given special advice (during home visits and monthly meetings) to ensure that they understood the importance of the cooking procedure used. Reports from random visits showed that compliance was good and in almost all that the oil was not being abused.

Materials

Red palm oil was provided by the Palm Oil Research Institute of Malaysia as the cooking oil Carotino. Sunflower oil pressed from locally produced sunflower seeds was purchased in Singida (HM Kassam, Singida, Tanzania). Rice was bought in the market in Singida. Ferrous sulfate, folic acid, mebendazole, and chloroquine were bought from a pharmaceutical retailer (MSD, Dar es Salaam, Tanzania). Pyrogallol, ascorbic acid, and butylated hydroxytoluene were purchased from Sigma Chemicals (Poole, United Kingdom). All other reagents and adsorbents were of analytic grade and were purchased from Merck Ltd (Lutterworth, United Kingdom). α-Carotene, β-carotene, and lycopene were purchased from Sigma Chemicals; *trans*-β-apo-10′-carotenal for the synthesis of the internal standard *trans*-β-apo-10′-carotenal oxime, lutein, zeaxanthin, and β-cryptoxanthin was from Hoffmann-La Roche Ltd (Basel, Switzerland). HPLC accessories (polyether ether ketone tubing, polyether ether ketone frits, and HPLC columns) were bought from Alltech (Carnforth, United Kingdom) and Phenomenex (Macclesfield, United Kingdom).

Antenatal care

All women in the study received hematinics, deworming supplies, and malaria prophylactics. From recruitment until delivery, ferrous sulfate (200 mg) and folic acid (5 mg) were given daily and chloroquine sulfate (2 × 250 mg) was given weekly. At recruitment, women were given a single dose of mebendazole (5 × 100 mg) as a treatment for acute or chronic hookworm, *Ascaris,* and *Trichuris* parasites. Schistosomiasis was not prevalent in the study area according to the district medical officer and thus medicine for treatment of this disease was not given.

Anthropometric measurements

Weight, height, and skinfold-thickness measurements were carried out at baseline and 1 and 3 mo postpartum as recommended (18). Four different skinfold-thickness measurements were taken in triplicate (biceps, triceps, subscapular, and suprailiac) and the equivalent fat content, as a percentage of body weight, was calculated by using tabulated values (19).

Collection and preparation of samples

Venous blood samples (5 mL) were collected at recruitment and 1 and 3 mo postpartum by venipuncture into evacuated tubes containing EDTA. Twenty minutes after collection, the blood samples (n = 237) were placed on ice until separated in the base laboratory at Singida College of Medical Laboratory Technology. After separation by centrifugation at 2000 × g for 10 min at room temperature, plasma samples were stored at −20°C until analyzed. Casual milk samples (40 mL) were collected 1 and 3 mo postpartum from both breasts by manual expression before and after the infants were fed on each breast. Attempts were made to collect equal amounts from each breast, which were then pooled. Milk samples were first collected in clear plastic beakers and then transferred to brown glass bottles to protect fat-soluble vitamins and carotenoids from light. All samples were collected during the morning between 1000 and 1200. The samples (n = 164) were put on ice and then stored at −20°C at the base laboratory in Singida. Samples were analyzed within 6 mo of collection. Milk and plasma carotenoids and retinol were measured by HPLC.

Hemoglobin measurement

Hemoglobin was measured at baseline and 1 and 3 mo postpartum by using the portable HemoCue device (HemoCue Ltd, Sheffield, United Kingdom), and values were compared with World Health Organization cutoffs (hemoglobin <110 g/L for pregnant women and <120 g/L for nonpregnant women). The performance of the HemoCue machine was monitored by measuring the hemoglobin concentration of the supplied standard cuvette after every 10 samples.

Determination of milk fat

Milk fat was determined by measuring the glycerol released by enzymatic hydrolysis of triacylglycerols with use of the diagnostic kit TRIG UNIMATE 5 (Hoffmann-La Roche) and a COBAS BIO centrifugal autoanalyzer (Roche Diagnostics, Basel, Switzerland). Milk samples were prepared for the automated analyses according to Lucas et al (20). The within- and between-assay CVs as determined by using pooled breast-milk samples were 1.4% and 4.1%, respectively.

HPLC analysis of plasma

The HPLC system from Shimadzu (Duisburg, Germany) comprised 2 LC-10AS delivery pumps, an SCL-10Avp system controller, an SIL-10Dvp autoinjector, an SPD-10Avp ultraviolet-visible light detector, and the CLASS VP software system for data acquisition. The mobile phase was degassed by using the vacuum degasser CSI6150. The column system, column temperature, and mobile phase were as described by Hart and Scott (21), except for the omission of butylated hydroxytoluene from the mobile phase. Samples were injected via the SIL-10Dvp autoinjector with a volume of 20 μL per sample and were held at 15°C in sealed vials to avoid evaporation and degradation. The peak response of retinol was measured at 325 nm with use of the ultraviolet-visible light detector; that of the carotenoids was measured at 450 nm after a wavelength change at 4.5 min.

The extraction procedure was a modification of the method described by Hess et al (22). After the plasma was thawed and mixed by vortex for 10 s, 200 μL was diluted with 200 μL distilled water and mixed with 400 μL ethanol. After the internal standard *trans*-β-apo-10′-carotenal oxime was added and mixed by vortex for 10 s, 1 mL hexane (including 0.05% butylated hydroxytoluene) for extraction was added and the mixture was mixed by vortex for another 20 s and centrifuged at 2000 × g for 5 min at room temperature. After separation, 700 μL hexane was transferred to a test tube, evaporated to dryness under nitrogen, and diluted with 200 μL mobile phase. Samples were then placed into sealed vials ready for injection. CVs were determined from pooled serum samples analyzed in conjunction

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TABLE 1
Daily consumption of carotenoids from red palm oil and sunflower oil

	Red palm oil[1]	Sunflower oil[1,2]
	μg/d (%)	*μg/d (%)*
Lutein	12 ± 6.0[3] (0.4)	5.4 ± 0.7 (50.0)
Zeaxanthin	9 ± 4.6 (0.3)	5.4 ± 0.7 (50.0)
β-Cryptoxanthin	9 ± 2.1 (0.3)	0.0 (0.0)
Lycopene	38 ± 19.1 (1.1)	0.0 (0.0)
all-trans-α-Carotene	909 ± 17.8 (26.0)	0.0 (0.0)
cis-α-Carotene[4]	389.1 ± 1.5 (11.1)	0.0 (0.0)
all-trans-β-Carotene	1114 ± 10.7 (31.9)	0.0 (0.0)
cis-β-Carotene[5]	1012.8 ± 9.1 (29.0)	0.0 (0.0)
Total provitamin A (*trans* isomers)	2034 (57.9)	0.0 (0.0)
Total carotenoids	3496 (100.0)	10.2 (100.0)

[1] Calculated amount in 4 plastic tablespoons of oil (12 g).
[2] Mean concentration of 6 samples analyzed in duplicate.
[3] $\bar{x}$ ± SD.
[4] Concentration calculated by using the extinction coefficient of *all-trans*-α-carotene.
[5] Concentration calculated by using the extinction coefficient of *all-trans*-β-carotene.

with the unknowns. The within-assay CVs for α-carotene, β-carotene, and retinol were 5.8%, 5.8%, and 2.6%, respectively. Between-assay CVs for these analytes were 13.9%, 14.7%, and 14.5%, respectively.

HPLC analysis of milk

The HPLC system from Waters (Milford, MA) comprised a dual-piston solvent delivery pump (model 600), a system controller (model 600), a photodiode array detector (model 996PDA), and the MILLENIUM software system for data acquisition (version 2010). The column system, column temperature, and mobile phase were as described by Hart and Scott (21), except for the omission of butylated hydroxytoluene from the mobile phase. Samples were injected manually via an injection valve (injector no. 7125; Rheodyne, Rohnert Park, CA), with a volume of 20 μL per sample. The peak response of retinol was measured at 325 nm; that of the carotenoids was measured at 450 nm. The extraction procedure was a modification of the method for breast-milk saponification from Craft Technologies, Inc (Wilson, NC). At the beginning of each day, a 5% (wt:vol) pyrogallol solution in ethanol containing ascorbic acid (0.5%, wt:vol) and a 40% (wt:vol) potassium hydroxide solution in methanol were prepared. The milk samples were thawed in a water bath at 45°C for 30 min, mixed by vortex, and homogenized before 1 mL was transferred into a 22-mL screw-top vial. To this solution were added 1 mL of a 5%-pyrogallol solution, the internal standard *trans*-β-apo-10′-carotenal oxime, and 2 mL of a 40%-KOH solution. This was then vortexed for 15 s, purged with nitrogen, and loosely capped before being ultrasonically agitated for 45 min at 45°C. After the sonication step, 2 mL distilled water and 2 mL hexane:ethyl acetate (90:10, by vol) were added and the sample was mixed by vortex for 15 s and then centrifuged at 2000 × *g* for 5 min at room temperature. The upper phase was transferred to a test tube and the extraction was repeated. The combined extract was washed twice with 2 mL anhydrous sodium sulfate (0.47 mol/L) and transferred to a clean tube for evaporation under nitrogen. The residue was redissolved in 200 μL mobile phase:ethyl acetate (80:20, by vol) ready for injection. CVs were determined from pooled breast-milk samples analyzed in conjunction with the unknowns. Within-assay CVs for α-carotene, β-carotene, and retinol were 5.2%, 3.5%, and 2.7%, respectively. Between-assay CVs for these analytes were 15.7%, 16.7%, and 14.7%, respectively.

Expression of milk carotenoid and retinol contents

The carotenoid and retinol contents of milk were calculated per kilogram milk fat (μmol/kg). The values were obtained by dividing both the carotenoid and retinol concentrations per liter (μmol/L) by the fat concentration (kg/L) of each sample. This was done because milk retinol expressed per milk fat was previously found to be a better indicator of maternal vitamin A status than was milk retinol concentration per volume (23). Potential variations related to differences in the milk-fat content of individual samples, which are unrelated to the vitamin A status of the mother, are therefore removed. According to World Health Organization criteria, values <28 μmol/kg milk fat were considered low (24). Breast-milk values ≥1.75 μmol/L (equivalent to ≥51 μmol/kg milk fat in this study) were considered to have normal vitamin A density (25).

HPLC analysis of dietary oils

Red palm oil and sunflower oil were analyzed according to Lietz and Henry (26) to ensure the quality and quantity of the micronutrients supplied. The results are presented in **Table 1**.

Data analysis

The data were double-entered into spreadsheets and cross-checked and analyzed by using SPSS 7.5 for WINDOWS (SPSS Inc, Chicago). Because plasma and milk carotenoids and retinol were log-normally distributed, analyses were conducted on log-transformed data. Geometric means and 95% CIs are presented. To determine dietary treatment effects, correlation analysis and repeated-measures analysis of variance (ANOVA) followed by the post hoc Dunnett's two-sided *t* test were performed.

RESULTS

Baseline characteristics

Characteristics of the study subjects are given in **Table 2**. Age, parity, time between pregnancies, height, and weight were not significantly different between the groups. Hemoglobin values were relatively high, apart from those in the sunflower oil group. The percentages of anemic pregnant women in the red palm oil, sunflower oil, and control groups were 14.8%, 39.3%, and 15.4%, respectively.

Compliance with supplementation and exclusions

Compliance with oil consumption was high. Only one woman declined to regularly consume the oil as requested. Two cases of over- or underconsumption were immediately addressed. One woman used parts of the supplied oil for frying but switched to the recommended food preparation method after consultation. Five volunteers were excluded from the study after enrollment for the following reasons: neonatal death (n = 2), not using the oil (n = 1), and moving to another village (n = 2). Data points for a few individuals in each study group were not recorded or were missing because of insufficient sample amounts, which accounts for the different sample sizes listed in the tables.

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TABLE 2
Characteristics of the study subjects[1]

	Red palm oil group	Sunflower oil group	Control group
Age of mother (y)	30.2 ± 1.4 [27]	27.2 ± 1.1 [29]	26.4 ± 1.3 [27]
Parity	4.5 ± 0.5 [27]	3.5 ± 0.4 [29]	3.2 ± 0.4 [27]
Time between pregnancies (y)	2.1 ± 0.1 [21]	1.8 ± 0.2 [22]	1.9 ± 0.2 [16]
Height (cm)	160.4 ± 1.3 [28]	159.1 ± 1.2 [29]	159.1 ± 1.1 [28]
Weight (kg)			
Third trimester	60.1 ± 1.5 [27]	60.1 ± 1.5 [28]	57.4 ± 1.3 [26]
1 mo postpartum	56.7 ± 1.6 [27]	56.3 ± 1.4 [29]	53.2 ± 1.2 [26]
3 mo postpartum	56.8 ± 1.7 [26]	56.0 ± 1.6 [27]	52.4 ± 1.3 [27]
Hemoglobin (g/L)			
Third trimester	120 ± 2.0 [27]	109 ± 2.8[2] [28]	121 ± 2.0 [26]
1 mo postpartum	135 ± 2.6 [27]	138 ± 2.4 [29]	135 ± 2.3 [26]
3 mo postpartum	134 ± 2.2 [26]	134 ± 1.5 [27]	134 ± 1.5 [27]

[1] $\bar{x}$ ± SEM; *n* in brackets.
[2] Significantly different from control group, $P < 0.01$ (Dunnett's two-sided *t* test).

Treatment effects on hemoglobin and parity

Hemoglobin values increased after parturition (Table 2). Only 0.3% of women in each group 1 mo postpartum and 0.5%, 0.5%, and 0.3% of women in the red palm oil, sunflower oil, and control groups, respectively, 3 mo postpartum were anemic. Parity correlated with both milk retinol (μmol/kg fat; $r = 0.394$, $P < 0.001$; $n = 79$) and plasma retinol (μmol/L; $r = 0.327$, $P < 0.01$; $n = 78$) 3 mo postpartum. After adjustment for parity by analysis of covariance, there was no significant difference in retinol concentration between the 3 treatment groups.

Milk fat

The mean milk-fat value in all women was 34 g/L throughout lactation, and 35, 35, and 33 g/L in women in the red palm oil, sunflower oil, and control groups 1 mo postpartum and 34, 33, and 34 g/L in women in these groups 3 mo postpartum. These concentrations are lower than previously reported milk-fat contents for European mothers of 35–51 g/L (27) and 41 g/L (28), but comparable with values found in Brazilian mothers of 30–39 g/L (29).

Plasma carotenoids and retinol

Measurements of plasma analytes were not significantly different between groups at baseline (**Table 3**). Plasma retinol concentrations indicated that a high proportion of women in all study groups were considered at moderate (0.71–1.05 μmol/L; 31 of 80) or high (<0.70 μmol/L; 20 of 80) risk of deficiency at baseline according to cutoffs defined for adults (3) (**Figure 1**). However, plasma retinol concentrations increased after parturition and the distribution changed to 56%, 63%, and 67% of women having plasma retinol concentrations >1.05 μmol/L 3 mo postpartum in the red palm oil, sunflower oil, and control groups, respectively (**Figure 2**).

At 1 and 3 mo postpartum, plasma α- and β-carotene concentrations were significantly higher in the red palm oil group than in the control group (Table 3). Values for α- and β-carotene were 42 and 3 times higher, respectively, in the red palm oil group than in the control group 1 mo postpartum and were 51 and 4 times higher, respectively, in the red palm oil group than in the control group 3 mo postpartum.

No significant differences in plasma retinol concentrations were observed between the study groups either 1 or 3 mo postpartum. Mean plasma retinol concentrations were >1.05 μmol/L throughout lactation and tended to be higher in the red palm oil group and control group than in the sunflower oil group 1 mo but not 3 mo postpartum (Table 3). Similarly, more women in the sunflower oil group tended to have plasma retinol concentrations <0.7 μmol/L 1 mo but not 3 mo postpartum. Plasma retinol

TABLE 3
Plasma retinol, α-carotene, and β-carotene at baseline (during the third trimester) and 1 and 3 mo postpartum[1]

	Red palm oil group	Sunflower oil group	Control group
		μmol/L	
Retinol			
Baseline	0.96 (0.85, 1.08) [27]	0.91 (0.77, 1.07) [28]	0.94 (0.81, 1.09) [25]
1 mo	1.29 (1.14, 1.47) [26]	1.14 (0.98, 1.33) [28]	1.40 (1.21, 1.62) [24]
3 mo	1.17 (1.01, 1.36) [25]	1.19 (1.03, 1.38) [27]	1.14 (0.94, 1.39) [27]
α-Carotene			
Baseline	0.01 (0.01, 0.03) [27]	0.02 (0.01, 0.04) [27]	0.02 (0.01, 0.03) [25]
1 mo	0.42[2] (0.35, 0.52) [26]	0.01 (0.01, 0.02) [28]	0.01 (0.00, 0.01) [23]
3 mo	0.51[2] (0.44, 0.58) [25]	0.01 (0.00, 0.01) [27]	0.00 (0.00, 0.01) [27]
β-Carotene			
Baseline	0.52 (0.41, 0.66) [27]	0.63 (0.50, 0.79) [27]	0.59 (0.49, 0.71) [25]
1 mo	0.77[2] (0.66, 0.91) [26]	0.31 (0.26, 0.36) [28]	0.26 (0.22, 0.31) [23]
3 mo	0.96[2] (0.86, 1.07) [25]	0.32 (0.25, 0.41) [27]	0.23 (0.17, 0.31) [27]

[1] Geometric $\bar{x}$ (95% CI); *n* in brackets.
[2] Significantly different from control group, $P < 0.001$ (Dunnett's two-sided *t* test).

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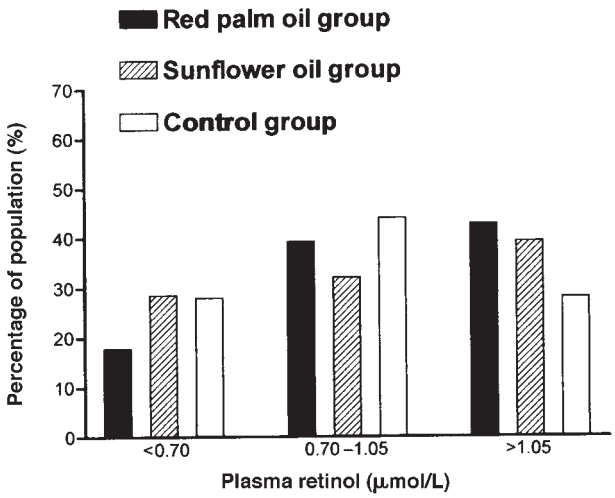

FIGURE 1. Plasma retinol distribution of the study population during the third trimester. n = 27, 28, and 25 in the red palm oil, sunflower oil, and control groups, respectively.

concentrations <0.7 μmol/L were found in 4%, 14%, and 4% of the women in the red palm oil, sunflower oil, and control groups 1 mo postpartum and in 12%, 11%, and 11% of the women in these groups 3 mo postpartum. Differences at 1 mo postpartum were not significantly different. No correlation was found between plasma hemoglobin and retinol concentrations for all women, and no significant differences in plasma retinol were observed between anemic and nonanemic women.

Milk carotenoids and retinol

Breast-milk α- and β-carotene concentrations per kilogram milk fat were significantly higher in the red palm oil group than in the control group 1 and 3 mo postpartum (**Table 4**). Values for α- and β-carotene were 56 and 3 times higher in the red palm oil group than in control group 1 mo postpartum and were 95 and 3 times higher in the red palm oil group than in the control group 3 mo postpartum.

The observed increases in α- and β-carotene concentrations did not lead to changes in milk retinol values in the red palm oil group (Table 4). However, a positive correlation between breast-milk β-carotene and retinol was found in the red palm oil group (r = 0.610, P < 0.001; n = 26). No correlation was found in either the sunflower oil group or the control group. More importantly, the breast-milk retinol concentration between 1 and 3 mo postpartum was almost maintained in both oil groups, but decreased in the control group (P = 0.055, interaction between all treatment groups and time, repeated-measures ANOVA). Changes in milk retinol concentrations were significantly different between the red palm oil and control groups (P = 0.041, Dunnett's two-sided t test; Table 4).

The percentages of women with low milk retinol concentrations of <28 μmol/kg milk fat 3 mo postpartum in the red palm oil, sunflower oil, and control groups were 12%, 11%, and 19%, respectively. The proportion of women with normal milk retinol density of ≥51 μmol/kg milk fat (equivalent to ≥1.75 μmol/L) 3 mo postpartum in the red palm oil, sunflower oil, and control groups were 27%, 37%, and 22%, respectively. The median, quartile, and extreme milk retinol concentrations per kilogram milk fat of all treatment groups 1 and 3 mo postpartum, respectively, are shown in **Figures 3** and **4**.

DISCUSSION

The results of this study indicate that supplementing pregnant women with red palm oil, which is rich in provitamin A, increases α- and β-carotene concentrations dramatically in both plasma and breast milk. Consumption of red palm oil did not appear to increase maternal plasma and milk retinol concentrations; however, consumption of either red palm or sunflower oil seemed to prevent the decrease in milk retinol concentrations that occurred in the control group between 1 and 3 mo postpartum.

Other studies in lactating women showed similar responses of β-carotene in plasma and breast milk after supplementation with either purified β-carotene (4, 8, 11), naturally occurring β-carotene (30), or red palm oil (7). In some of these studies, β-carotene supplementation either increased serum and breast-milk retinol concentrations after 3 mo (8) and 9 mo (4), or did not increase maternal serum and breast-milk retinol concentrations (7). We may not have observed an increase in either breast-milk or serum retinol concentrations in the red palm oil group in the present study for 2 reasons: *1*) the *all-trans*-β-carotene concentration given was not sufficient or *2*) the studied population was not vitamin A deficient. The present study provided a total of 184.8 mg *all-trans*-β-carotene as red palm oil (1.1 mg/d, given 7 d/wk for 24 wk), similar to the 210 mg purified β-carotene (3.5 mg/d, given 5 d/wk for 12 wk) provided by de Pee et al (8). However, 36% of the women in the population studied by de Pee et al had serum retinol concentrations <0.70 μmol/L, whereas in the present study only 4% of women in the control group 1 mo postpartum and 11% 3 mo postpartum had serum retinol concentrations <0.70 μmol/L. Detecting a significant change in retinol status is easier to achieve over a short study period when most of the subjects are classified as marginally to severely deficient. For this purpose, de Pee et al (8) chose anemic women as their study subjects because they were more likely to have low serum retinol concentrations. However, this strategy was not appropriate for our study population because there were few anemic women and, not surprisingly, no significant correlation between plasma hemoglobin and retinol.

Red palm oil is highly bioavailable, as shown in many previous studies (7, 13, 14, 31). The relative bioavailability of β-carotene in red palm oil is ≈10-fold greater than that in whole, uncooked

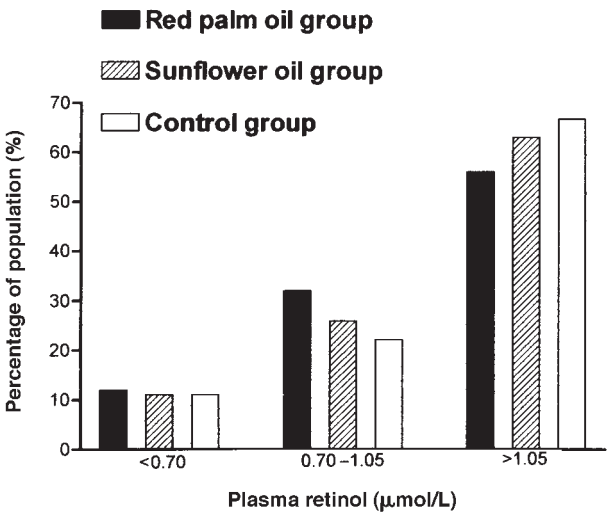

FIGURE 2. Plasma retinol distribution of the study population 3 mo postpartum. n = 25, 27, and 27 in the red palm oil, sunflower oil, and control groups, respectively.

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TABLE 4
Concentration of breast-milk retinol, α-carotene, and β-carotene 1 and 3 mo postpartum[1]

	Red palm oil group	Sunflower oil group	Control group
		μmol/kg milk fat	
Retinol			
1 mo	44.50 (38.87, 50.94) [28]	48.52 (40.98, 57.44) [29]	48.52 (41.34, 56.95) [27]
3 mo	44.64 (37.82, 52.68) [26]	46.09 (39.00, 54.47) [27]	38.45 (33.65, 43.93) [27]
α-Carotene			
1 mo	1.68[2] (1.40, 1.99) [28]	0.07 (0.02, 0.12) [29]	0.03 (0.00, 0.07) [26]
3 mo	1.90[2] (1.59, 2.23) [26]	0.01 (0.00, 0.03) [25]	0.02 (0.00, 0.05) [25]
β-Carotene			
1 mo	2.65[2] (2.27, 3.09) [28]	1.08 (0.85, 1.37) [29]	0.82 (0.66, 1.03) [26]
3 mo	3.00[2] (2.57, 3.49) [26]	1.17 (0.95, 1.43) [25]	0.89 (0.69, 1.13) [25]

[1] Geometric $\bar{x}$ (95% CI); *n* in brackets.
[2] Significantly different from the control group, $P < 0.001$ (Dunnett's two-sided *t* test).

carrots (32). Because the mass equivalence of β-carotene to retinol in raw carrots is 13:1 (33), the mass equivalence for red palm oil would be 1.3:1, which is the exact value found for β-carotene in oil by Hume and Krebs (34). However, this value should be used with extreme caution because the mass equivalence of red palm oil has not been tested with use of the new methods available (33).

Vitamin A intake and serum vitamin A concentrations during pregnancy influence the composition of breast milk. A study in Spanish women showed that women with retinol intakes <800 μg/d and serum retinol concentrations <1.05 μmol/L during the third trimester had lower breast-milk vitamin A values than did women with intakes and serum concentrations above these amounts (35). More importantly, weekly β-carotene supplementation during parturition increased the serum concentrations of retinol and carotenoids in a community trial in rural Nepal (36). Although no significant differences between the red palm oil and control groups were found in the present study either 1 or 3 mo postpartum, our finding of higher values of milk retinol in both oil groups 3 mo postpartum indicates that the oils might have an effect at a later stage in lactation. It was shown previously that breast-milk retinol concentrations respond slowly to daily β-carotene supplementation (4).

The relatively good bioavailability of β-carotene from red palm oil in our study could also be explained by the fact that all women were dewormed at the beginning of the study. *Ascaris* infection is known to be associated with reduced fat absorption in humans (37) and deworming results in improved utilization of β-carotene from the diet (38–40).

According to the World Health Organization (41), vitamin A deficiency is still a public health problem in many West African countries where red palm oil production is traditionally high. However, even if the oil is available, it does not necessarily suggest adequate and appropriate use or affordability. In Nigeria, red palm oil is used for seasoning foods and for deep-fat frying (42). Experiments with red palm oil repeatedly showed that only a small fraction of β-carotene is retained when the oil is used for frying (42–44). More importantly, experimental studies with frying oils showed that consumption of oxidized frying oils decreases plasma and liver vitamin A concentrations by as much as 50% (45, 46). On the other hand, conventional blanching and cooking has a negligible effect on the concentration of carotenoids in traditionally

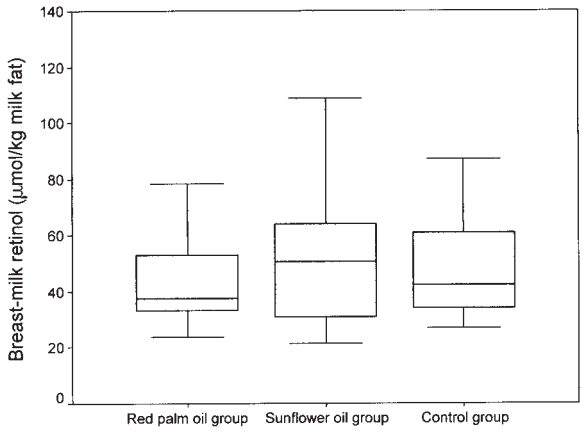

FIGURE 3. Boxplot summary of breast-milk retinol concentrations 1 mo postpartum. The box represents the distribution falling between the 25th and 75th percentiles, with the median as the horizontal line within the box. The vertical lines outside the box connect the largest and smallest values not categorized as outliers or extreme values. *n* = 28, 29, and 27 in the red palm oil, sunflower oil, and control groups, respectively.

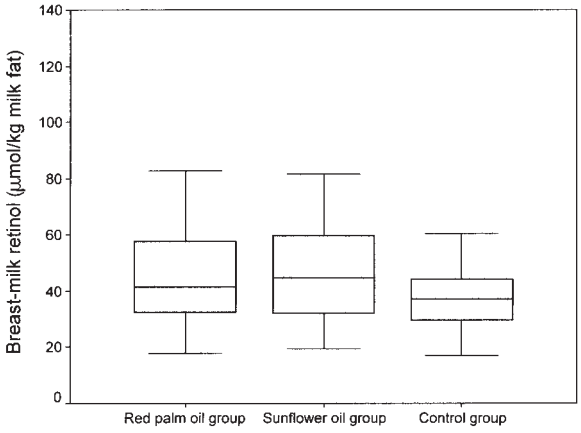

FIGURE 4. Boxplot summary of breast-milk retinol concentration 3 mo postpartum. The box represents the distribution falling between the 25th and 75th percentiles, with the median as the horizontal line within the box. The vertical lines outside the box connect the largest and smallest values not categorized as outliers or extreme values. *n* = 26, 27, and 27 in the red palm oil, sunflower oil, and control groups, respectively.

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used vegetables (47) and vitamin A–fortified soybean oil retains 100% of its biological value during cooking procedures requiring boiling at 100°C (45). It was for these reasons that women in the present study were advised to add the oil toward the end of the cooking process and to not use the oil for frying.

Both α- and β-carotene concentrations in plasma and breast milk increased dramatically in the red palm oil group over the duration of the study period, with the increase in plasma concentrations of α-carotene exceeding those of β-carotene. This finding agrees with the results of earlier studies in which palm-carotene-supplemented margarine (48) and palm oil carotenoids suspended in oil (49, 50) were given to healthy adults. The ratio of the final plasma and breast-milk concentration of α- to β-carotene was similar to the supplement used in this study and in a previous study using a palm oil carotenoid suspension in oil (50). However, the increases in plasma α-carotene concentrations in the red palm oil group were much higher (≈50 times) than those previously reported for healthy European volunteers [≈7–15-fold increase (48–50)]. This difference may be due to the very low plasma α-carotene concentrations of the volunteers at the beginning of the present study, which lay between the first and fifth percentiles of plasma α-carotene concentrations in American women of the same age group (51).

One of the most important findings of the present study is that consumption of red palm oil seems to retard the decline of retinol in breast milk during the progression of lactation. This agrees with results from Rice et al (4), who showed that β-carotene supplementation conserved breast-milk retinol up to 6 mo postpartum and increased it thereafter. The well-described decreases of milk retinol (3, 52) was apparent in the control groups of both studies. Assuming a similar kinetic of decay of milk retinol in the control group for 12 mo as described in an earlier study of nonprivileged Ethiopian women (52), and a stable milk retinol concentration in the red palm oil group, a child from the red palm oil group would receive 38% more retinol than would a child from the control group after 1 y of breast-feeding. With a presumed breast-milk consumption of 600 mL in the first 6 mo of life and 400 mL thereafter, the breast-milk retinol intake would be roughly 69-μmol higher in a child from the red palm oil group than in one from the control group. This amount would be equivalent to a high-dose vitamin A supplement of 104 μmol at 6 mo of age as recommended by the WHO/UNICEF/IVACG Task-Force (53), if one assumes that 90% of breast-milk retinol (54) and 50% of a high-dose vitamin A supplement (55) are absorbed. However, this calculation does not include the additional beneficial effect of elevated β-carotene concentrations in breast milk from women in the red palm oil group. Indeed, a recent study in Honduras showed that elevated β-carotene concentrations in breast milk alone resulted in a small but significant increase in infant serum retinol (7).

In summary, the results of the present study agree with earlier findings showing that red palm oil can increase maternal α- and β-carotene concentrations in both plasma and breast milk. More importantly, it seems possible to maintain breast-milk retinol concentrations for ≥3 mo after delivery through dietary supplementation with red palm oil. Both increases in milk β-carotene and maintenance of milk retinol concentrations are likely to be of considerable benefit to growing children. Although further testing is needed, recommendations to increase consumption of vegetable oil and to use mild cooking procedures should be considered for vitamin A–deficient pregnant and lactating women.

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