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ORIGINAL CONTRIBUTION

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Red palm oil in the maternal diet increases provitamin A carotenoids in breastmilk and serum of the mother-infant dyad

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Summary *Background* Despite vitamin A supplementation programs, vitamin A deficiency in children remains a public health concern in Honduras. *Aim of the Study* We investigated the effectiveness of short-term dietary supplementation of mothers with red palm oil as a strategy for improving the vitamin A status of the mother-infant dyad. *Methods* Lactating mothers in Colonia Los Pinos, a barrio of Tegucigalpa, Honduras, consumed a total of 90-mg β-carotene as red palm oil (n=32) supplements (n=36) or placebo (n=18) in six equal doses over 10 days. Carotenoids and retinol in maternal and infant serum, and breastmilk carotenoids and retinol were measured before and after supplementation. Maternal diet was evaluated by 24-hour recall. *Results* Maternal serum α-carotene and β-carotene concentrations were increased 2 fold by palm oil compared with 1.2 fold by β-carotene supplements. Changes were significantly different in infant serum α-carotene but not β-carotene among the three experimental groups. Increases in breastmilk β-carotene were greater for the palm oil group (2.5 fold) than for the β-carotene supplement group (1.6 fold) and increases in milk α-carotene concentrations (3.2 fold) were slightly greater than those of β-carotene. There were also small but significant changes among groups in breastmilk lutein and lycopene. Breastmilk retinol was not significantly different among the groups over the treatment period. *Conclusions* Red palm oil in the maternal diet increases provitamin A carotenoids in breastmilk and serum of the mother-infant dyad. The use of dietary red palm oil to improve the vitamin A status of this population should be further investigated.

Key words Red palm oil – β-carotene – Breastmilk – Carotenoids – Vitamin A

Introduction

In spite of numerous vitamin A supplementation programs over the past two decades, vitamin A deficiency remains a major concern for children in developing countries [1]. Therefore, we and others [2] have proposed that vitamin A supplementation programs alone will not be sufficient to solve the problem of vitamin A deficiency and that provitamin A carotenoids should be included in the diets of populations at risk for vitamin A deficiency. Our initial investigations of this hypothesis revealed that β-carotene supplementation of mothers consuming diets low in vitamin A increased serum and milk β-carotene in mothers and slightly increased serum retinol in their nursing infants [3]. Encouraged by these results, we have investigated the effect of β-carotene added to the diets of mothers as red palm oil or

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supplements on the vitamin A status of mothers and their nursing infants in a marginal barrio of Tegucigalpa, Honduras.

Subjects and methods

Study design

All protocols were approved by University of Arizona Human Subjects Committee prior to initiation of the project and were reviewed and agreed to by Honduran clinicians participating in the study. The sample size was determined as previously described to detect a difference of 25% between placebo and treatment groups [4]. Ninety-eight mother-infant pairs were recruited from the well-baby clinic in the Colonia of Los Pinos, Tegucigalpa, Honduras. Prior to sample collection, all mothers signed Informed Consent Forms in Spanish in accordance with regulations of the University of Arizona and with requirements of the Honduran Ministry of Health. Mothers were informed in detail in Spanish about the study including risks/benefits before signing Informed Consent Forms. Since the protocol of the mRDR (modified dose response) assay requires that 4–5 hours elapse between the administration of the loading dose [5] and acquiring the blood sample, vitamin A_2 was administered to mothers and infants immediately upon registering in the clinic. A Spanish-speaking field nurse obtained a health history for mothers and infants and 4th-year medical students completed a 24-hr dietary recall for mothers. Mothers were then provided breakfast with supplements.

Ninety-eight mother-infant pairs were randomly assigned to one of three groups. Mothers in Group I received 90 mg β-carotene as red palm oil concentrate. Mothers in Group II received capsules containing 90 mg purified β-carotene (BASF) and Group III mothers received placebo capsules identical in appearance to those containing β-carotene. The total dosage of 90 mg β-carotene was based on our previous study showing a significant increase of maternal milk and serum following short-term supplementation of mothers with that dosage[3]. Mothers returned on days 3, 5, 7 and 9 for an additional supplement (β-carotene, palm oil or placebo), breakfast and gifts. On day 10, the protocol for day 1 was repeated for both mothers and infants, except that no dietary or health histories were obtained.

Anthropometrics

Gravidity, parity, days of lactation, and age of mothers were self-reported. Weights of mothers and infants together and mothers separately were determined using a bathroom scale, and weight of the infant determined by subtraction. Heights of mothers were measured without shoes.

Treatments

mRDR

A loading dose (0.35 μmol/kg body weight) of the vitamin A analog, 3,4 didehydro-retinyl acetate (vitamin A_2) was administered to mothers and infants as previously described [5]. A single blood sample was obtained from both mothers and infants 4.5 hours after administration of the analog. To maintain the appropriate time difference between sampling, both blood samples were taken from participants in the same order.

Supplements

All supplements were administered in the clinic under supervision of the field staff. Palm oil, β-carotene supplements or placebo capsules were provided to mothers with a breakfast meal which contained ~ 8 g fat. The meal consisted of tortillas, black beans, crema (a local dairy product similar to sour cream) and 8 oz. whole milk. A powdered preparation of β-carotene (BASF, 15 mg) was dispersed in cornstarch and packaged in an opaque capsule. Because no more than 15 mL (equivalent to 15 mg β-carotene) red palm oil fraction could be mixed in a serving of beans without producing off-color, six treatments of 15 mg each were needed to provide a total of 90 mg. One serving spoon (~ 15 mL) of red palm oil was mixed with a serving of black beans (~ $^3/_4$ cup). This provided ~ 15 mg β-carotene and 6.8 mg α-carotene. Mothers in the placebo group received the same meal and a capsule containing only cornstarch but otherwise identical to the β-carotene-containing capsule.

Dietary Analyses

A 24-hr dietary recall was administered to mothers by 4th medical students who had been instructed in use of the instrument by the field physician. Mothers were asked to report serving sizes in volumes, pieces or weight. For vegetables, milk, ice cream, beans, rice or pasta, one serving was considered one-half cup. One piece of fruit, one pat margarine, or 2–3 oz meat was considered a serving. Data collected reflected intake 24 hours prior to the clinic visit.

Collection of Samples

At mid-morning, mothers provided milk (5–10 mL) samples by manual expression with supervision from a

field physician or nurse. At the time of predicted peak appearance of vitamin A_2 in serum, 4 to 6 hours after administration [5], blood samples were taken by venipuncture from mothers and infants. To minimize stress, only one attempt to obtain blood from infants was made.

Subject remuneration

While in the clinic, mothers were given lectures and literature on health and nutrition in Spanish language by our field team and were provided gifts (e. g., soaps, lotions, jewelry, cosmetics, etc.) at each clinic visit immediately prior to leaving the clinic. The total value of gifts for mothers who completed the study was approximately $30 (US). At the conclusion of the study, certificates of participation with names inscribed were given to each mother.

Sample handling

Blood was maintained at ambient temperature in the field in coolers protected from the light, (< 6 hrs) and transported to the Department of Clinical Laboratories, Hospital-Escuela in Tegucigalpa. Serum was prepared by centrifugation (600 × g, 5 min, 25°C), portioned into 1–2 mL samples and stored at –20°C. Milk samples were maintained in opaque, capped plastic scintillation vials at –4°C in coolers in the field, transported to the laboratory and stored at –20°C. At the end of the study, frozen milk and serum samples were transferred to coolers, packed in antibacterial ice-packs (Microban) and hand-carried to Arizona by one of us (LC). Immediately upon arrival in Tucson, samples were delivered to the laboratory and stored at –70°C until they were analyzed. Serum and milk samples were analyzed batchwise and were complete within 6 and 9 months, respectively from the date they were obtained. To control for artifacts due to sample storage and transport, blood samples were donated by the Principal Investigator in the field, analyzed on return, and compared with her blood samples obtained in Tucson.

Analysis of serum retinol and carotenoids

Carotenoids and retinol were extracted from serum as previously described [4] and analyzed within 4 hours.

Analysis of milk retinol and carotenoids

Milk samples were hydrolyzed as previously described [6]. To calibrate the method and to control for sample degradation during processing and storage, samples from a milk pool obtained from Tucson mothers just prior to initiation of the study were analyzed with each batch of milk samples.

Quantitation of milk lipids

Milk lipid concentrations were determined by "creamatocrit" as previously described [7]. Data are the average of three determinations from a single sample.

HPLC Analysis

Extracted milk and serum samples were analyzed using two Waters model 510 pumps (Waters Associates, Milford, MA), a Beckman 520 autosampler with a 50 µL-loop (Beckman Altex, Palo Alto, CA), a Milton Roy model SM4000 programmable UV/VIS detector (Milton Roy, Riviera Beach, FL), and a Waters Maxima 820 chromatography workstation equipped with a 5-µM YMC C_{18} reversed-phase column (4.6 × 250 mm) as previously described [6]. Components were eluted with 95 % solvent A (ACN:THF, 85:15, v/v) with 250 ppm BHT and 0.05 % TEA) and 5 %, solvent B (50 mmol/L ammonium acetate in methanol with 0.05 % TEA) at a flow rate of 2.5 mL/min. Total time for a single HPLC analysis was 13 min. The same HPLC column was used throughout the study.

Quantitation

The HPLC was calibrated at the beginning of the study using standard curves constructed from authentic β-carotene, α-carotene, β-cryptoxanthin, lycopene, lutein, and retinol obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, Standard reference material 968 b). Recovery of retinol and carotenoids was determined by internal standardization as previously described [6]. Limits of detection for retinol and carotenoids were estimated at 0.003 and 0.02 µmol/L respectively using a signal to noise ratio of 3:1 Carotenoids and retinol were quantitated using the appropriate extinction coefficients as described previously [8].

Analysis of carotenoids in supplements

By HPLC analysis, we determined that the red palm oil fraction contained 1 mg β-carotene/mL. β-Carotene and α-carotene were present at a ratio of 0.55-mg β-carotene/0.25-mg α-carotene. No other carotenoids were detected. Provitamin A activity of α-carotene in palm oil was not considered in calculations. β-Carotene

concentrations in the BASF preparation was verified by HPLC [9]. No carotenoids other than β-carotene were detected.

Materials

All chemicals were technical grade or better and were obtained from Aldrich (Milwaukee, WI) or Sigma Chemical Co (St. Louis, MO). Solvents for chromatography were HPLC grade from Burdick Jackson (Muskegon, MI). Ethanol was from Quantam Chemical Corp., USI Division (Tuscola, IL). The red palm oil fraction was provided by the Palm Oil Research Institute of Malaysia (PORIM).

Data Analysis

Data were expressed as means ± SEM. Differences among the three experimental groups before and after supplementation were analyzed by one-way analysis of variance (ANOVA) using STATA (College Stn, TX). ANOVAs were calculated controlling for baseline concentrations and were considered statistically significant at $P < 0.05$.

Results

Anthropometrics – (Table 1)

The average BMI of the mothers was within US guidelines for normal women [9]. Approximately 20% of the mothers had BMI values consistent with poor nutrition, (< 18.5), however neither serum retinol nor β-carotene concentrations were associated with BMI indices. Thirteen of the mothers self-reported low birth weight infants (< 2.5 kg). Six infants were between 12 and 24 months of age; the remainder was between the ages of one and 12 months.

Table 1 Anthropometric data for study population

	Mean + SD	Range	*n*
Mothers			
Age (y)	26.0 ± 6.5	15–43	97
Height (m)	1.6 ± 0.1	1.3–1.7	91
Weight (kg)	53.6 ± 10.0	36.3–80.7	97
BMI (kg/m^2)	22.3 ± 4.3	14.5–35.9	91
Gravidity	3.9 ± 2.5	1–13	83
Parity	3.4 ± 2.1	1–11	83
Infants			
Age (mo)	7.0 ± 4.0	1–24	95
Weight (kg)	7.8 ± 2.6	3.6–18	97

Compliance and follow-up rates

A total of 85 mothers and 76 infants completed all phases of the study. Thirteen mothers did not participate on both study days because of illnesses or family responsibilities. Twenty-two infants were lost to the study either because we were unable to obtain sufficient blood for analysis on a single venipuncture or because of objections of the parents to venipuncture on more than one day.

Dietary analyses

Only foods containing significant sources of vitamin A were included in the 24-hour recall (Table 2). Patterns of food consumption were comparable among treatment groups. Because the Honduran sugar supply has been fortified since 1993 with retinyl palmitate (6.6 mg/kg) [10], sugar, soft drinks, and purchased sweets were included as vitamin A-containing foods. Diets consisted mainly of rice, beans and tortillas. By self-report, twenty-four (26%) of the women consumed no fruits or yellow and green vegetables, and 54 (57%) had one serving or less. Moreover, 76 (81%) reported no intake of foods high in β-carotene, 17 (18%) reported one serving of foods high in β-carotene, and only one reported more than one serving. Similarly, 33 (35%) recalled no dairy products and 65 (69%) recalled one serving or less. In contrast, only 15 (16%) consumed no meat and 79 (84%) reported consuming one or more servings of meat over the previous 24 hours. Only 9 (10%) of the mothers consumed no foods prepared with oil and 34 (36%) reported 3 or more servings in the past 24 hours.

Table 2 Qualitative analysis of diet[1]

No. servings	No. mothers reporting[2]						
	0	1	2	3	4	5	>6
Fruits and vegetables[3]	24	30	17	14	4	4	1
Fruits and vegetables high in β-carotene[4]	76	17	1	0	0	0	0
Dairy products[5]	33	32	18	9	2	0	0
Meat, eggs, fish[6]	15	42	24	5	6	1	1
Foods prepared with oil[6]	9	23	28	17	8	5	4
Sugar[7]	66	23	5	0	0	0	0

[1] n=94
[2] Number of times mothers reported consuming foods in any quantity during the previous 24 h.
[3] All green and yellow vegetables
[4] Foods providing ~1 mg/serving: carrots, ayote, mango
[5] Margarine, butter, crema, milk, ice cream, cheese
[6] Fried, stir-fried or braised in shortening or oil
[7] Sweet rolls, cookies or soft drinks. One tsp. refined sugar contains ~ 25 µg retinol (see text)

Initial concentrations of carotenoids and retinol

Serum

Initial concentrations of maternal and infant serum carotenoids were highest for β-carotene and lowest for β-cryptoxanthin (Tables 3, 4). Concentrations of one or more of the pro-vitamin A carotenoids (β-cryptoxanthin, α-carotene β-carotene) were below our levels of detection for at least 10 infants on one or more sampling days. The average initial serum retinol concentrations of all infants was 0.67 μmol/L and was unrelated to age (r = 0.18). Five children were vitamin A deficient (serum retinol < 0.35 μmol/L) and serum retinol concentrations of approximately half the children were < 0.7 μmol/L. Comparison of serum carotenoids and retinol of the Principal Investigator from blood samples obtained in the field and those obtained in Tucson revealed no significant degradation due to transport.

Table 3 Response of serum carotenoids to maternal supplementation with red palm oil or β-carotene

Treatment group	n[1]	Lutein/Zeaxanthin	β-Cryptoxanthin	Lycopene	α-Carotene	β-Carotene
		μmol/L ± s. e. m.				
		Mothers				
Palm oil						
Baseline	31	0.117 ± 0.04	0.040 ± 0.01	0.175 ± 0.08	0.086 ± 0.03	0.322 ± 0.39
Supplement		0.117 ± 0.04	0.036 ± 0.01	0.159 ± 0.07	0.244 ± 0.10[2]	0.654 ± 0.06[2]
β-Carotene						
Baseline	36	0.131 ± 0.04	0.039 ± 0.02	0.195 ± 0.08	0.067 ± 0.03	0.207 ± 0.01
Supplement		0.118 ± 0.05	0.036 ± 0.02	0.168 ± 0.07	0.069 ± 0.03	0.257 ± 0.02[3]
Placebo						
Baseline	18	0.130 ± 0.03	0.047 ± 0.03	0.225 ± 0.08	0.076 ± 0.03	0.267 ± 0.12
Supplement		0.110 ± 0.03[2]	0.041 ± 0.02	0.182 ± 0.06	0.073 ± 0.04	0.295 ± 0.16
		Infants				
Palm oil						
Baseline	28	0.090 ± 0.04	0.025 ± 0.01	0.054 ± 0.03	0.032 ± 0.01	0.137 ± 0.09
Supplement		0.082 ± 0.04	0.027 ± 0.01	0.068 ± 0.04	0.049 ± 0.03[4]	0.155 ± 0.10
β-Carotene						
Baseline	28	0.084 ± 0.04	0.029 ± 0.01	0.068 ± 0.03	0.036 ± 0.02	0.148 ± 0.08
Supplement		0.090 ± 0.04	0.029 ± 0.02	0.056 ± 0.03	0.039 ± 0.02	0.131 ± 0.10
Placebo						
Baseline	10	0.098 ± 0.04	0.035 ± 0.01	0.067 ± 0.02	0.043 ± 0.02	0.149 ± 0.05
Supplement		0.091 ± 0.05	0.032 ± 0.01	0.081 ± 0.05	0.031 ± 0.02	0.167 ± 0.10

[1] Completed all phases of the study
Differs significantly from baseline concentration compared to placebo using Anova; [2] *P < 0.0001;* [3] *P < 0.001;* [4] *P < 0.05*

Table 4 Response of serum retinol, to maternal supplementation with red palm oil or β-carotene

Treatment group	Mothers		Infants	
	Retinol μmol/L ± s. e. m.	Vitamin A_2/A_1[1]	Retinol μmol/L ± s. e. m.	Vitamin A_2/A_1[1]
Palm oil	(n = 32[2])		(n = 26[2])	
Baseline	1.34 ± 0.04	0.009 ± 0.008	0.64 ± 0.03	0.024 ± 0.02
+ 10 d	1.41 ± 0.06	0.013 ± 0.020	0.74 ± 0.04	0.022 ± 0.03
β-Carotene	(n = 36[2])		(n = 20[2])	
Baseline	1.28 ± 0.05	0.012 ± 0.010	0.71 ± 0.06	0.04 ± 0.030
+ 10 d	1.20 ± 0.05	0.010 ± 0.001	0.74 ± 0.04	0.01 ± 0.001
Placebo	(n = 18[2])		(n = 11[2])	
Baseline	1.42 ± 0.08	0.014 ± 0.010	0.67 ± 0.08	0.05 ± 0.04
+ 10 d	1.47 ± 0.08	0.014 ± 0.010	0.71 ± 0.09	0.03 ± 0.02

[1] μmol/L serum didehydroretinol ÷ μmol/L serum retinol
[2] Completed all phases of the study

Milk

Carotenoids and retinol are presented as simple concentrations (μmol/L), and to minimize intra-and inter-individual variability [11], corrected for total milk lipid (μmol/g lipid) as well (Table 5). Milk β-carotene was weakly associated with serum retinol (r = 0.3) but not serum β-carotene of infants.

Response to supplementation

Serum carotenoids (Table 3)

Changes in maternal serum concentrations before and after supplementation between the three groups were significantly different for α-carotene and β-carotene but not for the other carotenoids using ANOVA. Changes in maternal serum α-carotene and β-carotene concentrations increased significantly ($p < 0.0001$) more for the palm oil supplement group compared with the β-carotene supplement and placebo groups. These differences remained even after controlling for baseline concentrations of α-carotene and β-carotene respectively and were normally distributed with general bell-shaped distributions. Serum α-carotene concentrations were significantly increased in serum of infants in the palm oil group compared to the other treatment groups ($P < 0.05$). Changes in other infant serum carotenoids were not significant among the three treatment groups.

Serum retinol (Table 4)

Changes in maternal serum retinol concentrations were near significance among groups following supplementation with red palm oil ($P < 0.07$) but were clearly not different following β-carotene supplementation ($P < 0.73$). Although serum retinol of infants increased relative to initial concentrations in response to maternal supplementation with red palm oil ($P < 0.05$), when compared to changes in the other treatment groups using ANOVA, these changes missed statistical significance ($P < 0.06$) by our criteria. Similar results were obtained when only infants with initial serum retinol concentrations < 0.7 μmol/L were considered. (ANOVA, $P < 0.07$). Ratios of neither maternal nor infant serum vitamin A_2 (3,4 didehydro-retinyl acetate) to vitamin A_1 (retinol) were significantly different following supplementation of with β-carotene or red palm oil indicating no effect on liver stores.

Milk- (Table 5)

Changes in milk concentrations of α-carotene ($P < 0.01$) and β-carotene ($P < 0.02$) before and after supplementation were significantly different between the three experimental groups using ANOVA. These differences remained after controlling for the baseline values for α-carotene and β-carotene respectively. Increases in β-carotene concentrations were greater for the palm oil group (2.5 fold, $p < 0.0001$) than for the β-carotene sup-

Table 5 Response of milk retinol and carotenoids to maternal supplementation with red palm oil or β-carotene

Treatment group	n[1]	Retinol	Lutein	β-Cryptoxanthin	Lycopene	α-Carotene	β-Carotene
		μmol/L	*nmol/L ± s. e. m.*				
Palm oil							
Baseline	29	1.63 ± 1.01	16.34 ± 7.20[2]	7.39 ± 4.30	21.71 ± 13.06[2]	10.53 ± 4.25[4]	35.33 ± 15.53[4]
+ 10 d		1.10 ± 0.41	17.87 ± 5.39	8.64 ± 2.49	23.46 ± 11.12	33.41 ± 12.60	88.40 ± 37.65
β-Carotene							
Baseline	32	1.28 ± 0.59	15.26 ± 6.97	5.69 ± 2.52	19.14 ± 8.71	12.38 ± 6.34[3]	38.90 ± 17.26[3]
+ 10 d		1.28 ± 1.37	18.87 ± 7.02	6.80 ± 2.12	20.97 ± 9.62	18.74 ± 13.41	64.09 ± 31.97
Placebo							
Baseline	18	1.95 ± 0.87	18.64 ± 7.52	8.45 ± 3.10	16.27 ± 5.92	8.62 ± 3.44	31.74 ± 12.60
+ 10 d		1.09 ± 0.63	12.17 ± 4.28	5.82 ± 2.16	11.31 ± 5.06	10.41 ± 6.04	36.33 ± 18.17
			nmol/g lipid ± s. e. m.				
Palm oil							
Baseline	29	0.04 ± 0.01	0.41 ± 0.24	0.18 ± 0.09	0.53 ± 0.27	0.26 ± 0.15	0.89 ± 0.51
+ 10 d		0.03 ± 0.02	0.50 ± 0.24	0.25 ± 0.16	0.72 ± 0.62	0.96 ± 0.66	2.42 ± 1.26
β-Carotene							
Baseline	32	0.05 ± 0.04	0.59 ± 0.46	0.21 ± 0.17	0.72 ± 0.56	0.46 ± 0.37	1.54 ± 1.16
+ 10 d		0.04 ± 0.02	0.72 ± 0.37	0.28 ± 0.19	0.87 ± 0.65	0.90 ± 0.92	2.78 ± 2.38
Placebo							
Baseline	18	0.06 ± 0.04	0.71 ± 0.48	0.29 ± 0.16	0.86 ± 0.45	0.41 ± 0.21	1.37 ± 0.97
+ 10 d		0.03 ± 0.02	0.43 ± 0.27	0.20 ± 0.12	0.39 ± 0.28	0.35 ± 0.24	1.21 ± 0.80

[1] Completed all phases of the study
Differs significantly from baseline concentrations compared to placebo; [2]$P < 0.01$; [3]$P < 0.001$; [4]$P < 0.0001$

plement group (1.6 fold, $p < 0.006$) relative to placebo. Similarly, increases in milk α-carotene concentrations in the palm oil group (3.2-fold) were greater than those in the β-carotene group (1.6-fold). There were also small but significant changes among groups in milk concentrations of lutein ($P < 0.001$) and lycopene ($P < 0.01$) after controlling for baseline concentrations. When expressed relative to milk lipid, changes in milk retinol concentrations were not significantly different among the treatment groups.

Discussion

Short-term dietary supplementation of mothers with red palm oil significantly increased α-carotene and β-carotene concentrations in maternal serum and breastmilk. Increases in infant serum α-carotene ($P < 0.05$) but not β-carotene, were also significant among the treatment groups. Since the red palm oil fraction we used contained almost twice as much β-carotene as α-carotene, these results suggest that either β-carotene was preferentially metabolized to retinol by mothers and/or infants or that α-carotene is more bioavailable from red palm oil than β-carotene. In either case, the present data suggest that red palm oil when consumed in the maternal diet might improve vitamin A status in the maternal-infant dyad.

We saw no increases in maternal serum or milk retinol in response to β-carotene or palm oil supplementation. In agreement with our results, pregnant women in rural Tanzania supplemented with 4 tablespoons red palm oil daily for three months had significant increases in α-carotene and β-carotene but not retinol in serum [12]. Similar results were seen in Bangladeshi mothers receiving 7.8 mg β-carotene/day for 9 months [18]. All of these studies used purified β-carotene supplements. Conversely, Indonesian women who received an enriched wafer containing 3.5 mg β-carotene/day for 12 weeks showed significant increases in both serum and milk retinol [2]. It is noteworthy that this wafer contained fat, iron, vitamin C, folic acid and other nutrients that could have enhanced carotenoid bioavailability. These data point out the need for studies investigating the dietary factors influencing carotenoid bioavailability.

In agreement with our previous study [3] and that of others [2], changes in other serum carotenoids were not significant following either supplementation with β-carotene or red palm oil. Some workers have reported effects of β-carotene supplementation on concentrations of other serum carotenoids [13, 14]. However, because of the large intra- and inter-individual variability, small changes in serum and milk carotenoid concentrations should be interpreted with caution. In fact, as we have previously pointed out [4], sample sizes of > 100 per group could be required to document statistically significant changes of 25% in β-carotene concentrations between two groups.

All initial maternal serum carotenoids were substantially lower than those of U. S. mothers [9, 15, 16]. However, β-carotene concentrations were about twice those of Indonesian (0.19 μmol/L) [2] and Jakartan (0.18 μmol/L) [17] women.

The few available studies in children indicate that serum carotenoid concentrations increase with age. In the only study of newborns of which we are aware [19], median serum β-carotene concentrations were 0.014 μmol/L (n=22) at birth, rising to 0.044 (n=18) at 2–6 weeks of age. Infants in the present study had average serum β-carotene concentrations of 0.14 μmol/L), about half those of Guatemalan preschoolers [20] while in older children in Belize, Japan and France, serum β-carotene concentrations were similar to those of adults (0.5–0.7 μmol/L) [21, 22]. These data suggest that infants and young children may have low carotenoid reserves. Because low carotenoid status could impact vitamin A stores and therefore increase risk for infection, further studies are needed to investigate age-related carotenoid status.

Infant serum retinol concentrations were slightly increased, but in contrast to our previous study [3], not significantly, relative to placebo following maternal β-carotene supplementation. Our two studies cannot be directly compared, since our pilot study did not include a placebo group, and used a water-miscible preparation instead of the powdered preparation used here. A likely explanation of these results is lower bioavailability of the supplements used in this study compared with the water-miscible beadlets used in our previous work [3]. It is noteworthy that breastfed Bangladeshi infants of mothers receiving 7.8 mg β-carotene daily for 6 months showed a slight increase in serum retinol concentrations by six months, which similarly to our results, was not significant when compared to the placebo group [18]. As discussed above, β-carotene supplements may not be sufficiently bioavailable to impact vitamin A status in dietary amounts.

The small but significant increases in breastmilk lutein and lycopene in response to palm oil supplementation could be explained by dietary fluctuations. The large increase (1.5 fold) in milk α-carotene following supplementation with β-carotene capsules, however, was unexpected and we can offer no biological explanation for these results. We are unaware of studies of effects of β-carotene supplementation on milk carotenoids other than our previous one [3]. In this study as well, milk α-carotene was increased following β-carotene supplementation. In our studies, the HPLC peaks representing milk α-carotene and β-carotene were well resolved. Moveover, as discussed above, serum α-carotene was not increased in mothers supplemented

with β-carotene. These two observations argue against artifactual inflation of the milk α-carotene peak by co-eluting isomers of β-carotene or oxidation products, however, this explanation cannot be ruled out in the absence of chemical characterization of the eluent. Carotenoid-carotenoid interactions are complex and poorly understood. Intensive study will be required to predict their effects *in vivo*.

In agreement with our previous study [9] milk retinol concentrations were not significantly increased following either β-carotene or palm oil supplementation in spite of significant increases in milk β-carotene. Conversely, Indonesian women who consumed wafers containing 3.5 mg β-carotene/day for 12 weeks significantly increased both serum and breastmilk retinol concentrations. In this same study, however, women consuming 3.5 mg β-carotene/day as vegetables had no increases in either serum or milk retinol concentrations. Again, these data point out the importance of understanding the factors influencing the bioavailability and absorption of β-carotene supplements.

Composition data for carotenoids is limited for Honduran foods and consumption of foods high in carotenoids was minimal. Further, the accuracy of a food frequency questionnaire is questionable in this population due to their lack of familiarity with this manner of describing the diet. Therefore, we reasoned that a qualitative assessment would be the most appropriate. As shown in Table 2, diets were very low in fruits and green vegetables. Therefore, although these women consumed only modest amounts of vitamin A, most of it was preformed retinol from animal sources or fortified foods and not from pro-vitamin A carotenoids. Although oil is used for cooking, oils are typically animal fats ("lard"). However, the high consumption of foods prepared with oils suggests that red palm oil is potentially an effective vehicle for increasing vitamin A intake in this population of mothers.

Average initial serum retinol concentrations of the infants in our study barely achieved the 0.7 μmol/L WHO minimum recommendations. In agreement with recent national surveys in Honduras [10], approximately half the children had serum concentrations below this value and five were vitamin A deficient. On average, mothers reported one or two episodes of diarrhea per month. Since infection and diarrhea result in transitory depletion of vitamin A [23], the initial vitamin A status of these children indicates that they are at risk for infection, with infection in turn further lowering vitamin A stores. Therefore, measures to increase pro-vitamin A carotenoids such as the red palm oil vehicle described here should be further investigated.

In summary, palm oil supplementation of mothers substantially increased concentrations of pro-vitamin A carotenoids in serum and milk of Honduran mothers. The maternal diet was low in vitamin A and at least half the infants in this study were at risk for vitamin A deficiency. Where red palm oil is part of the usual diet, even diets that are nutrient deficient, vitamin A deficiency is rare [24, 25]. Palm oil is an effective energy source, and does not increase the risk for heart disease [24, 26–28]. Since foods prepared with oil are a major component of the local diet, dietary red palm oil has the potential to significantly improve their vitamin A status. Strategies to introduce high carotene oils into the diet of this population should be further investigated.

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