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EFFECT OF β-CAROTENE SUPPLEMENTATION IN HONDURAN LACTATING MOTHERS ON SERUM β-CAROTENE AND RETINOL LEVELS OF THEIR BREAST-FED INFANTS

by

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STATEMENT BY AUTHOR

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This work is dedicated to my son,

Zunayed Kabir

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ABSTRACT

The objective of this study is to assess the effects of pure β-carotene supplementation in the lactating mothers on serum β-carotene and retinol levels of their breast-fed infants. The relevant data of this study are taken from a nutritional intervention trial conducted in Tegucigalpa, the capital city of Honduras, participated by forty five healthy, lactating mothers and their infants. During the intervention trial, all of the mothers took 30 mg of pure β-carotene capsules with their morning meal for three alternate days (a total of 90 mg) in the week-long trial. The mothers provided 10 milliliter of breast milk per sampling and 5 milliliter of blood per draw. Two milliliter of blood was collected from each infants during the trial. β-Carotene and retinol concentrations were quantitated in the breast milk and in the serum samples using high-performance-liquid-chromatography (HPLC). The data received from the HPLC analysis were analyzed by a parametric method (*t-test*) using a two-sided test with the significance level, α=0.05. Results of the *t-test* confirmed that the supplementation of β-carotene to the lactating mothers has a significant effect on their breast-fed infants' serum retinol concentrations. This is reflected in the increased levels (by a ~10 fold) of infants' serum retinol concentrations (p<0.001). It is also observed that there is a statistically significant increase of the β-carotene levels in the maternal breast milk and serum samples due to this β-carotene supplementation. A similar increase of retinol levels in the maternal blood samples is observed. In addition, a modest correlation (r=0.43) between the retinol concentrations of

post-supplemented maternal breast milk and the post-supplemented serum of infants, equal to or less than four months of age, is also observed. This dose-response relationship suggests that a regular supplementation of β-carotene to the lactating mothers might help their breast-fed children in harnessing the long term beneficial effects of vitamin A.

CHAPTER ONE

INTRODUCTION

1.1: Introduction

Vitamin A is a fat soluble vitamin. It is an essential micronutrient in many stages of the human lifecycle. It has critical roles in reproduction, immune system, maintenance of cellular differentiation and formation of specific glycoprotein. Therefore, it is one of the required elements for maturation of mucous membrane, linear growth and development of a growing infant (3,4,8,15,22,25). At human infancy, mother's breast milk is the only source of dietary vitamin A in the form of *retinol*. Children with mild vitamin A deficiency are at two to three times the risk of infection and four times the risk of dying, compared to children with adequate vitamin A levels (28).

1.2: Vitamin A Deficiency

Vitamin A deficiency is one of the major public health problems for infants and small children of the developing countries. Vitamin A deficiency is the most common cause of irreversible childhood blindness. Numerous studies (4,6,14,18,19,22,24) confirmed that, as many as 250,000-500,000 pre-school children may develop xerophthalmia (permanent loss of vision) per year due to direct or indirect vitamin A deficiency. It has also been reported that marginal vitamin A deficiency (0.07 μmol/gm of liver) (40) is associated with increased risk of various infections such as, diarrhea,

gastroenteritis, severe post-measles symptoms that contribute significantly to childhood morbidity and mortality (1,2,4,5,13,22). Breast milk from a lactating mother in the Third World countries may be the only protective factor against these diseases including onset of night blindness, conjunctival xerosis and xerophthalmia among the vitamin A deficient children (3,4,6,25,27).

1.3: Natural Sources of Vitamin A

We know that dark green leafy vegetables are the best source of dietary vitamin A. Usually, it is found as pro-vitamin A (α-carotene, β-carotene, β-cryptoxanthin) in amaranth, spinach and broccoli and in yellow-orange fruits and vegetables such as, carrots, pumpkin, mangoes, papaya, apricot etc. In ester form, vitamin A is present in animal products like liver, egg yolk, butter, cheese, oily fish, and meat (22). Vitamin A also can be formed in vivo from pro-vitamin A such as, β-carotene, α-carotene, β-cryptoxanthin. β-Carotene is first converted to retinal and then from retinal to retinol (8,15,22,28). Excess amount vitamin A as retinol stores in the liver for future use in any diseases.

1.4 Background and Significance

An accurate detection of low vitamin A is a crucial task. Plasma retinol level <0.35 μmol/L (<0.7 μmol/L) sometimes is not a reliable indicator of low vitamin A status of an individual (30); and the relative-dose-response (RDR) test is not a practical test to conduct in a regular screening programs in many regions of the World to diagnose

(4,9,22,24). It is necessary to address that vitamin A requires certain amount of dietary fat, protein and zinc to get absorbed, transported and utilized in metabolic reactions (3,7,26,28,32,34,35). The consumption of these essential elements by women of child bearing age in the Third World countries is mostly from local plant sources. Sometimes the amount of β-carotene consumption is affected by cultural norms, season and the household's economic condition. Unfortunately, most women can not overcome these barriers to fulfill their recommended daily dietary requirements of vitamin A.

As it has been mentioned earlier, breast milk is the only potential source of vitamin A for many infants around the World. Until now, approximately, twelve retinyl esters have been identified in mature breast milk. Among these esters, retinyl palmitate and retinyl stearate are the most predominant retinoids in human milk, and they provide ~60% of retinoids through the breast milk. This has a significant impact on the health of the children of the Third World countries. We believe that an adequate supplementation of vitamin A for these mothers during their last trimester of pregnancy or in the early lactational period may be a sustaining source of vitamin A in their breast milk. It is also a cost-effective measure to reduce morbidity and mortality among infants and young children (4,7,22) of Third World countries.

The recommendations from FAO/WHO for daily requirements of vitamin A are: 250-400 μg/day for children, approximately 750 μg/day for adults and 850 μg/day for

The recommendations from FAO/WHO for daily requirements of vitamin A are: 250-400 μg/day for children, approximately 750 μg/day for adults (males and females) and 850-1250 μg/day for lactating women (22,28). It is necessary to indicate that the amount of β-carotene supplement depends on the amount consumed in each meal. However, it is suggested that at least 6 mg of β-carotene per day should be consumed by an adult (39).

During pregnancy, the plasma vitamin A concentration decreases an average 25% . The International Vitamin A Consultative Group and the World Health Organization recommend that no more than 3000 μg RE (<10,000 IU) vitamin A be given daily. One should be very careful about the dose of a vitamin A supplement because a very high dose (>10,000 IU) of supplementation during pregnancy may have teratogenic effects on the fetus (22,23,33,41). However, in populations with low levels of vitamin A consumption, the risk of such effects is minimal.

β-Carotene is considered as to be the most important carotenoid in terms of human nutrition. It is an effective carotenoid, because ingested β-carotene can be converted in human body by a normal metabolic process into two molecules of vitamin A, whereas all other carotenoids (pro-vitamin A) can form only one vitamin A. Also, it has additional biochemical functions such as, singlet oxygen quenching and antioxidant activities, and is safer than vitamin A. We chose to use pure β-carotene as supplement in

this population, because some lactating women were marginally deficient; and some of them might have become pregnant during their lactational period.

1.5 Hypothesis

β-Carotene supplementation of lactating mothers will significantly increase their breast-fed infants' serum retinol concentrations.

1.6 Objectives /Aims

To determine the effects of β-carotene supplement on β-carotene and retinol concentrations in a population of vitamin A marginal-to-deficient lactating mothers and their infants in:

(1) maternal serum and breast milk

(2) infant's serum.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Introduction

Forty-five Honduran lactating mothers received 30 mg purified β-carotene in capsule form for 3 alternate days (a total of 90 mg) in a week long trial with the morning meal (usually, tortillas, beans and 'mantequilla'- buttery spread) (32). Retinol and β-carotene concentrations were quantitated in all samples using high-performance-liquid-chromatography (HPLC).

2.2 Study Population

The targeted subjects were Spanish-speaking lactating mothers living around the capital city of Honduras, Tegucigalpa- a heterogeneous population with ethnic origins in Asia, Africa, Germany and Spain. The local diet is chiefly comprised of tortillas, eggs, beans, plantains and rice. The socio-economic status (SES) of this population is low, and the people are poorly educated.

2.3 Sample Size

Since, our primary aim was to observe the serum retinol level in infants after a supplement with pure β-carotene to the lactating mothers, we used available data of serum retinol in mothers and infants to calculate the required sample size. The

indicated that a sample size of 33 lactating mothers were needed to observe a statistically significant difference in serum and milk retinol, before and after supplementation of β-carotene. The sample size was estimated using the statistical program, STATA (37) with the percent difference between means and the standard deviations of serum retinol (μ=1.42; std $\pm$0.5) from the pilot study in Honduras (1991) and from the normal lactating well nourished women in Tucson (μ=1.61; std $\pm$0.17). The sample size for the infants was calculated separately. Both calculations were done assuming one sided test (positive increase) at the significance level, α=0.05 and power=95%. This study had high power for the final sample size of 44 pair of subjects (mother-infant).

2.4 Subject Consent

All enrolled women signed Informed Consent Forms in accordance with regulation of the University of Arizona, Tucson, USA and the University of Honduras, Tegucigalpa, Honduras. All of the participants were informed in detail in Spanish about the study including risks/benefits before signing the consent forms.

2.5 Recruitment

Recruitment of the subjects was done by medical students from the University of Honduras under the supervision of a physician in the Department of Community Health at the main teaching hospital, Hospital Escuela, Tegucigalpa, Honduras. They were successful in recruiting and maintaining subject compliance. Subjects were recruited

from the local "well-baby" clinics (free-clinics) in the marginal Barrios surrounding the capital city of Honduras, Tegucigalpa.

2.6 Inclusion/Exclusion criteria

Forty-five lactating mothers and their nursing infants were recruited into the study. All babies were full-term. Their age at the time of entry into the study ranged from 12 days to 24 months. No infants were known to have congenital or chronic diseases. Also, none were known to receiving vitamin A supplements. Mothers were aged 15 - 45 yrs., parity ≤ 5, non-pregnant, non-smoking, were not suffering from any chronic disease or infection, and were not consuming vitamins. Subjects who did not meet the inclusion criteria above, who were severely malnourished, who declined to participate for the duration of the study, or who declined to sign an Informed Consent Form were excluded at the beginning of the study.

2.7 Compliance

The compliance of the mothers was excellent (>98%). Forty-four out of 45 women and 32 out of 45 (71%) infants participated in the whole study. Free meals and small gifts were made available to all children in the family at the end of the study.

2.8 Anthropometric Measurements

Data on maternal height and current weight (an effect modifier) were collected during the interviews. Weight before and during the last pregnancy at term was recorded using self-reporting questionnaire.

2.9 Study Design

The study was a week long nutritional intervention trial. A total of 90 mg pure β-carotene was supplemented to the study subjects every other day for 3 days.

2.10 Sample Collection

Blood samples (5 ml/draw) of these mothers were collected on day 1 (two samples; one before and one 5 hr. after suppl.), and on days 2, 3 and 4. Breast milk (10-15 ml/sample) of the mothers and blood samples (2 ml/draw) of the babies were collected at the beginning (day 1) and at the end of the study (day 5) (Appendices A-1 and A-2).

2.11 Sample Preparation

Milk and Serum: The procedure for milk and serum sample preparations have been described in detail elsewhere (10, Appendices B and C).

2.12 Materials

β-Carotene capsules were obtained from Hoffmann-La Roche. Methanol, hexane, and tetrahydrofuran (THF) were purchased from Baxter (Muskegon, MI) and ethanol was

from Quantum Chemical (Tuscola, IL). All solvents used for HPLC were HPLC grade or better. Organic solvents for HPLC were filtered through a 0.45 mm fluoropore filter (Millipore, Bedford, MA) prior to use.

2.12.1 Standardization - External standards were utilized for the quantitation of carotenoids in mature breast milk. Standards of retinol and β-carotene were prepared by serial dilution into HPLC mobile phase from respective stock-retinol and β-carotene solutions containing approximately 1 mg/ml solubilized in THF + 0.5 g/l BHT.

2.12.2 High Performance Liquid Chromatography (HPLC) - The requirements for the procedure were a single HPLC pump and an absorbence monitor. In Dr. Canfield's laboratory, HPLC analysis was performed using a Waters pump (Model 510), a Programmable Detector (Milton Roy; Model SM 4000), a 50 µl loop and system controller (Waters Maxima 820; version 3.02; Waters Assoc., Milford, MA). A YMC (Moris Plains, NJ) reverse-phase C-18 column, 5 micron, 120A ODS column (4.6 mm x 250 mm) was used for all analyses. Carotenoids were isocratically eluted with running solvent consisting of 10% THF, 90% methanol and 0.5 g/l butyrated hydroxytoluene (BHT) at a flow rate of 1.6 ml/min β-carotene was detected at 452 nm and retinol at 325 nm. Total time for a single analysis including re-equilibration of the column is 20 min.

2.13 Data Management

All data were recorded onto pre-coded forms. Data were entered and cleaned using spreadsheet (MS-Excel; Ver. 4.0; 1993), then it was saved as an "ASCII" file to convert into statistical software STATA for analysis (37).

2.14 Statistical Method

2.14.1 Analysis with continuous variables

Mother:

- Serum β-carotene and retinol increases with supplement: pre-supplement sample, S_1 and post-supplement sample, S_5 (2 comparisons).
- Breast milk β-carotene and retinol increase with supplement: post-supplement samples, S_2 and S_5 (2 comparisons).

Infant:

- Infant's serum β-carotene and retinol increase with maternal supplement: post-supplements, S_2 and S_5 (2 comparisons).

Here a total of six statistical analyses was needed to meet the specific aims of the study (Tables 2,3,4).

2.14.2 Multiple logistic regression

- Multivariate logistic regression analyses would be performed to observe whether the maternal age and number of pregnancies influenced on the infant's blood level of β-carotene and retinol.

Cut-off values for deficient groups of mothers and infants were defined in logistic regression analyses as if their baseline serum levels of β-carotene and retinol were ≤25%. Then an increase was defined as if any of these values had gone up more than 25% of the baseline value after the supplementation.

2.14.3 Analyses with descrete variables

We hypothesize that the mothers who are severely vitamin A deficient do not have the ability to transfer β-carotene and/or retinol to their infants through the breast milk. The severely deficient mothers' livers will utilize most of the supplements. To determine this occurrence, we categorized our study participants into two different serum level groups. Therefore, a stratified analysis (using linear regression) was done to test the effect of baseline vitamin A status on infant's serum β-carotene and retinol levels (<0.26 μmol/L vs. ≥0.26 μmol/L and <1.83 μmol/L vs. ≥1.83 μmol/L respectively) post-supplementation (S_5) (12).

CHAPTER THREE

RESULTS

Forty-five Honduran lactating mothers were enrolled in the study. The characteristics of normal lactating mothers is presented in Table 1. To analyze all six comparisons of β-carotene and retinol levels, a parametric method (t-test) was used with a two-sided test with significance level, α=0.05. A non-parametric test (Signrank) was used to verify the test results. In all instances the results of the non-parametric test were found to be confirmatory.

β-Carotene supplementation in mothers positively affected their serum level of vitamin A. Table 2 depicts the comparisons at varying intervals of β-carotene levels in mothers' serum before and after supplementation with purified β-carotene- all 3 comparisons were statistically significant. A significant increase (α<0.05) in serum β-carotene levels was found with the increasing days of β-carotene supplementation (sample 4=0.88 μmol/L and sample 5=1.01 μmol/L ; 13% increase). However, for retinol, the effect of β-carotene supplementation on mothers' serum was less evident. Only the 5th sample was statistically significantly higher than the first sample (9% increase; $p <$ 0.01).

The influence of β-carotene supplementation on the concentration of β-carotene and retinol in breast milk was analyzed using the samples 2 and 5 - the only samples that were available. With this limitation, β-carotene breast milk concentration was positively significant at the level of α=0.05 (p<0.001) affected by β-carotene supplementation. For the samples obtained during the period of intervention, retinol concentrations were not altered by the β-carotene supplementation (Table 3).

For infants, the positive effect of maternal supplementation is reflected in their serum levels of retinol rather than their serum β-carotene levels (p<0.001 and p=0.32 respectively, Table 4). These results likewise were consistent in both the parametric and non-parametric tests.

The possible influence of mothers' age and/or the number of pregnancies on pretreated serum and breast milk retinol levels were studied using both univariate and multivariate logistic regression analyses. The univariate logistic regression model failed to detect any statistically significant effects of either the age or the number of pregnancies on the pre-treated serum or breast milk concentrations. However, the multivariate logistic regression analysis shows that the number of pregnancies of the mother is borderline statistically significant negative factor related to her baseline serum retinol level when adjusted for age (p=0.08 < p=0.1) (Tables 5 and 6).

Mothers were categorized as either deficient or not with regards to β-carotene and retinol. The categorization was done based on mothers' serum β-carotene and retinol levels at the time of their entering into the study. These were labeled as low in β-carotene and in retinol, when the corresponding levels were <0.26 μmol/L and <1.83 μmol/L, respectively; and high in β-carotene and in retinol, if the corresponding levels were >0.5 μmol/L and >2.69 μmol/L respectively (12). Linear regression analyses indicated that the maternal baseline serum value does not have any significant influence on infants' serum levels of β-carotene and retinol (p =0.79 in both cases).

CHAPTER FOUR

DISCUSSION

4.1 Introduction

Absorption of β-carotene and retinol depends on various external and internal factors. Bioavailability of β-carotene, among other factors is affected by: (i) absorption modifiers (e.g. fat) (8,32,36), (ii) nutritional status of the individual (e.g. an adequate serum retinol has an inhibitory effect on the enzyme that cleaves carotene into retinol) (20,22,34); (iii) host related factors (e.g. intestinal parasites, malabsorption etc.) (15,22,30,31,42). Also, carotene absorption requires not only a sufficient amount of dietary fat but also a sufficient amount of protein and zinc (3,26,34,35). It is possible that some or all of these factors might have influenced the outcome of this study and may have prevented us from observing a clear positive correlation between supplementation in mothers and changes in offsprings' vitamin A status.

4.2 Sample size

Calculations were performed to reveal that 33 mother-infant pairs (with 95% power) would be needed to observe the statistically significant results. Forty-five pairs were enrolled in the study (36% more than required). A significant number of blood samples of infants and a few samples of breast milk were lost during collection, preservation and preparation.

4.3 Sample preparation

Breast milk contains an average of 4.5 g/dl fat, approximately 2 fold more fat than that in colostrum. To measure the carotenoids in mature milk, saponification is a necessary step to release the carotenoids from the lipid matrix in the mature breast milk. Without this step, it is not possible to detect carotenoids in mature breast milk (22). In the process of saponification, KOH was used in this study (10). KOH is a chemical agent which is highly unstable and might lead to incomplete saponification of the fat globules in the milk. However, internal laboratory control suggested that the overall recovery of β-carotene and retinol in milk was satisfactory.

4.5 Diet and Dietary fat

As mentioned earlier, pro-vitamin A or retinol is a fat soluble vitamin which requires an adequate amount of dietary fat to achieve at least ~50% absorption of total consumption (22,26). In this study, a food-frequency-questionnaire (FFQ) was not administered therefore dietary fat was not assessed. β-Carotene and retinol both are highly dependent on an adequate quantity of dietary fat and proper intestinal absorption (6,14,15,22,26). Moreover, one of the many studies has suggested that high fiber diet can lead to carotenoid-malabsorption (22). In this study, no specific restrictions on high fiber diet were imposed. We have since developed guidelines and restrictions for a regular diet during the subsequent studies. A large age range among the infants (minimum=12 days; maximum=2 years) might also have confounded the study due to the unrestricted toddlers' diet.

4.6 Intestinal Parasites

Several studies have suggested that the *Ascaris lumbricoides* (round worm) impairs the absorption of retinol in both adults and children and probably leads to the depletion of liver storage (16,30,31,43,44,45). Interestingly, one study has demonstrated that *Ascaris* actually accumulates retinol in their internal structure from their host's intestine (44). This study has reported that fecal losses due to malabsorption and/or blood loss into colon or rectum from the pathological effects of the helminth on the intestinal mucosa affect the serum levels of retinol in humans. In the present study, it is observed that 43% of the enrolled mothers had *Ascaris lumbricoides* infections. This might have influenced our study results (breast milk retinol levels) by interfering the metabolic process of β-carotene. (Table 8).

4.7 Statistical analysis

In this study, the data were analyzed in several steps. Some of the analyses did not show any statistically significant results. Therefore, in our study, both simple and multiple logistic regression were used in order to elucidate any possible influences of mothers' age, number of pregnancies on pre-treated (baseline) serum and milk retinol levels.

Due to the unavailability of the data of maternal body weight, it was not possible to examine the influence of their body weight on serum and/or milk β-carotene and retinol levels (32). The regression analysis with infants' body weight has shown a

statistically significant effect on serum retinol and none of the other factors had any significant effect. The potential influence of an infant's age, birth weight and sex on their pre-supplementation retinol and β-carotene serum levels can also be important (22,32,34). However, this issue is left for future investigation.

We did not observe any strong correlation between the concentration of β-carotene in breast milk and infants' serum β-carotene or in retinol (r=0.19 and r=0.05 respectively) (Figs. 2,3,4,5). However, when the association between post-supplement breast milk and post-supplement serum of infants under or equal to 4 months in β-carotene and retinol levels was considered, a strong correlation is observed. β-Carotene of post-supplemented mothers was negatively correlated whereas, for retinol a satisfactory correlation exists between mothers' breast milk retinol and serum retinol in infants of $\leq$4 months in post-supplement status (r=-0.21 and r=0.43 respectively) (Fig. 6).

4.8 Blood collection in infants

As discussed in the methods section, a sufficient amount of blood per sample is needed for the exhaustive carotenoid extraction method which requires a minimum 50 µl of serum per chromatography. In our study, less than optimal blood collection procedures in infants were a major factor. Fairly an adequate number of infants' blood samples could not be utilized due to insufficient quantity. Hemolysis of red blood cells (RBC) in infants was another problem in the blood-sample collection.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

β-Carotene supplementation in Honduran lactating mothers positively affected their serum and breast milk levels of vitamin A. This increase was positively reflected in infants' serum retinol levels.

- We have observed a positive increase in serum β-carotene and retinol levels in mothers after supplementation with 30 mg of β-carotene for 3 alternate days. A significant increase ($\alpha<0.05$) in serum β-carotene levels was found with the increasing days of β-carotene supplementation. No significant changes were observed in breast milk retinol levels. This result was consistent in both the parametric and non-parametric tests.

- The positive effect of maternal supplementation is reflected in their infant's serum levels of retinol rather than their serum β-carotene levels ($p<0.001$ and $p=0.32$ respectively). In addition, we have found that the post-supplemented serum retinol levels of infants of ≤4 months old is correlated with maternal post-supplemented breast milk retinol levels ($r=0.43$).

- The univariate logistic regression model failed to detect any statistically significant effects of either the age or the number of pregnancies on the pre-treated serum or breast milk concentrations. However, the multivariate logistic regression analysis shows that the number of pregnancies of the mother is a borderline statistically significant negative factor related to a lower level of pre-treated serum retinol when adjusted for maternal age (p=0.08 < p=0.1).

The results of this study support that the women in developing countries are the targeted population for vitamin A supplementation as a long term preventive strategy for reducing Global morbidity and mortality among small children due to hypovitaminosis A (4,18,21,25,27,28,29,38).

5.2 Recommendation

The eradication of vitamin A deficiency should have a high priority on the Global health strategies, since it contributes to the premature death of millions of infants and young children annually. The following steps should be taken in order to accomplish this by targeting the host, the diet and the environment:

- Nutritional and general health education to encourage to increase the consumption of vitamin A rich products by utilizing existing infrastructure. An increased

consumption from a natural dietary source is safe and cost-effective for any individual at any stages of human life.

- Fortification of food staples: The fortification of locally available foods (dried milk, salt, sugar, oil, margarine etc.) is strongly recommended for prolong period of time for those countries where nutritional blindness among children is highly prevalent (13,16,17,18,28). However, all fortified foods should be made available and easily accessible for the target population.

- Supplementation of vitamin A orally or intramuscularly to the severely deficient groups of people on the basis of regular health screening procedure.

- More research to map the prevalence of vitamin A deficiency regionally and seasonally can contribute to more efficient prevention programs.

All underprivileged women and mothers, whose habitual intakes are near basal requirement should have an additional 100 μg retinol equivalents (RE) during pregnancy and 300 μg RE during lactation (22,28).

Table-1: Characteristics of normal Honduran lactating mothers.

Characteristics	Ave. ± std*	n*
Age, yrs.	22.9 ± 6.26	44
No. of pregnancy	3.02 ± 1.96	44
Parity, numbers	2.91 ± 1.84	44
Length of breast-feeding, months	9.91 ± 6.76	44
Feq. of breast-feeding, times/day	7.14 ± 3.23	44
Baseline breast milk (μmol/L):		
β-Carotene	0.02 ±0.01	33
Retinol	1.78 ± 1.14	36
Baseline serum (μmol/L):		
β-Carotene	0.09 ± 0.05	44
Retinol	1.58 ± 0.38	44

*No. of observation; ℵ=Average ± standard deviation.

Table-2: Mean ($\bar{x}$), standard deviation (±std), sample size and the results from the t-test in maternal serum β-carotene and retinol levels.

	β-Carotene (μmol/L)				Retinol (μmol/L)			
Sample	$\bar{x}$	±std	n$^{\Phi}$	*t-test*	$\bar{x}$	±std	n$^{\Phi}$	*t-test*
1st	0.09	0.05	44		1.58	0.38	44	
2nd	0.11	0.06	43	***	1.6	0.39	43	ns
1st	0.09	0.05	44		1.58	0.38	44	
5th	1.01	0.48	42	***	1.67	0.40	42	**
4th	0.88	0.38	43		1.63	0.37	43	
5th	1.01	0.48	42	**	1.67	0.40	42	ns

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ and "ns" = not significant.
Φ= Expressed in numbers.

Table-3: Mean ($\bar{x}$), standard deviation (±std), sample size and the results from the t-test in maternal breast milk β-carotene and retinol levels.

	β-Carotene (μmol/L)				Retinol (μmol/L)			
Sample	$\bar{x}$	±std	n$^{\Phi}$	*t-test*	$\bar{x}$	±std	n$^{\Phi}$	*t-test*
2nd	0.02	0.01	33		1.78	1.4	36	
5th	0.1	0.09	32	***	1.69	0.95	32	ns

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ and "ns" = not significant.
Φ= Expressed in numbers.

Table-4: Mean ($\bar{x}$), standard deviation (±std), sample size and the results from the t-test in infants' serum β-carotene and retinol levels.

	β-carotene (μmol/L)				Retinol (μmol/L)			
Sample	$\bar{x}$	±std	n$^{\Phi}$	*t-test*	$\bar{x}$	±std	n$^{\Phi}$	*t-test*
2nd	0.05	0.04	28		0.89	0.27	32	
5th	0.05	0.03	30	ns	1.02	0.24	31	***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ and "ns" = not significant.
Φ= Expressed in numbers.

Table-5: Univariate and multivariate logistic regression analyses regarding the influence of the number of pregnancies on baseline serum retinol levels after adjusting for maternal age.

Independent Variables	Univariate			Multivariate		
	OR✧	95% CI♌	*p**	OR✧	95% CI♌	*p**
Maternal age	1.05	0.86-1.28	0.65	1.42	0.90-2.25	0.13
No. of pregnancies	0.84	0.51-1.37	0.49	0.42	0.16-1.11	0.08

Dependent variable: maternal serum retinol levels.
✧= odds ratio; **p*-value in Wald's statistics; ♌= 95% confidence interval.

Table-6: Univariate and multivariate logistic regression analyses regarding the influence of the number of pregnancies on baseline breast milk retinol levels after adjustimg for maternal age.

Independent Variables	Univariate			Multivariate		
	OR✧	95% CI♌	*p**	OR✧	95% CI♌	*p**
Maternal age	1.05	0.86-1.28	0.65	1.11	0.75-1.64	0.59
No. of pregnancies	0.83	0.51-1.37	0.49	0.74	0.28-1.96	0.55

Dependent variable: breast milk retinol levels.
✧= odds ratio; *=*p*-value in Wald's statistics; ♌= 95% confidence interval.

Table 7: Intestinal parasitic infections among normal Honduran lactating mothers and their infants.

Type	Mothers (n=28)	Percent (%)	Infants (n=23)	Percent (%)
Ascaris lumbricoides	12	42.9	3	13
Necator americanus	2	7	2	8.7
Trichuris trichiura	1	3.5	0	0
None	13	46.5	16	69.6
Other	---	---	2	8.7

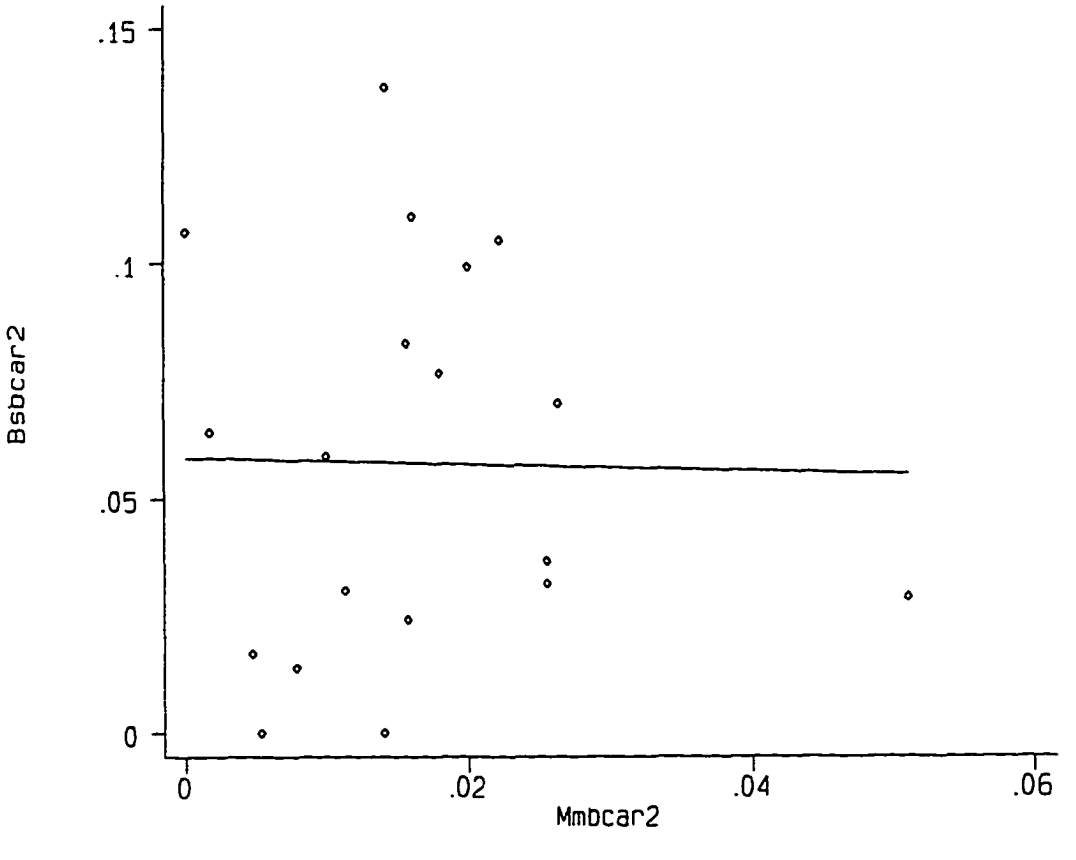


Figure 1: Correlation between maternal baseline breast milk β-carotene (μmol/L) and infants' baseline serum β-carotene levels (μmol/L) (n=19); Mmbcar2 = Maternal breast milk β-carotene levels and Bsbcar2 = Infants' serum β-carotene levels at baseline.

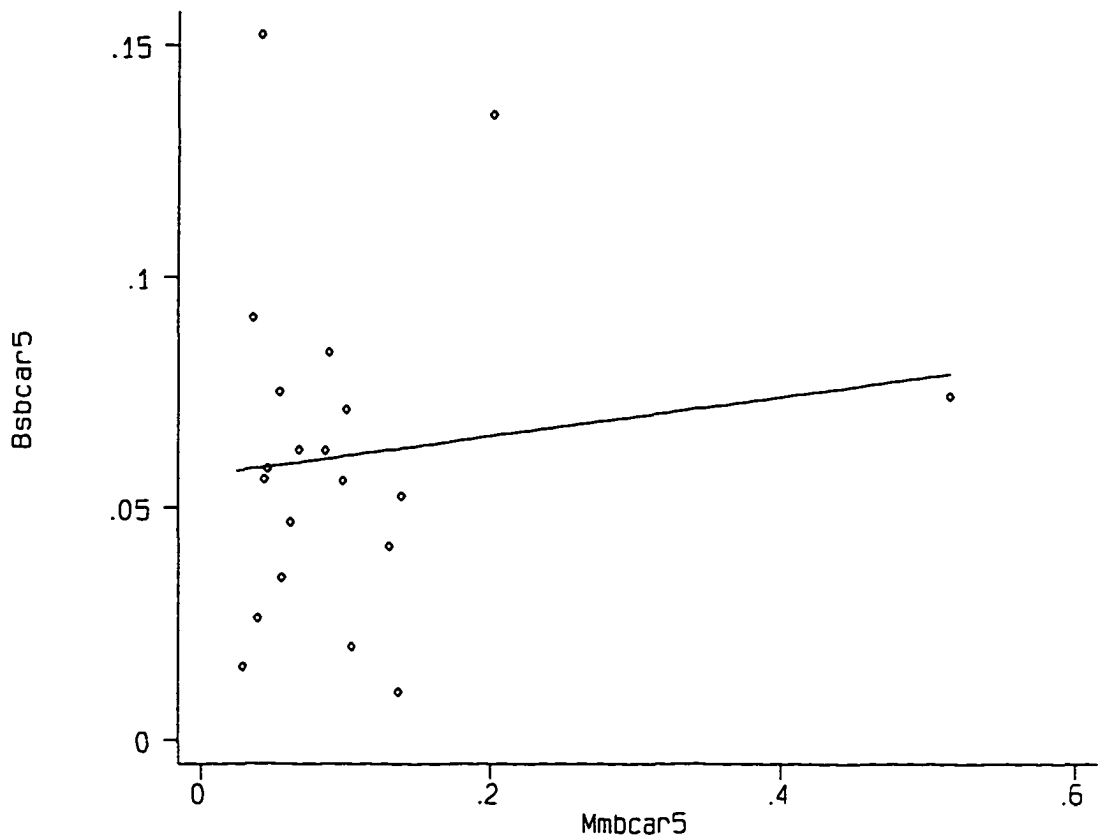


Figure 2: Correlation between maternal breast milk β-carotene and infants' serum β-carotene levels (μmol/L) after supplementation (n=20); Mmbcar5 = Maternal breast milk β-carotene levels and Bsbcar5 = Infants' serum β-carotene levels after supplementation.

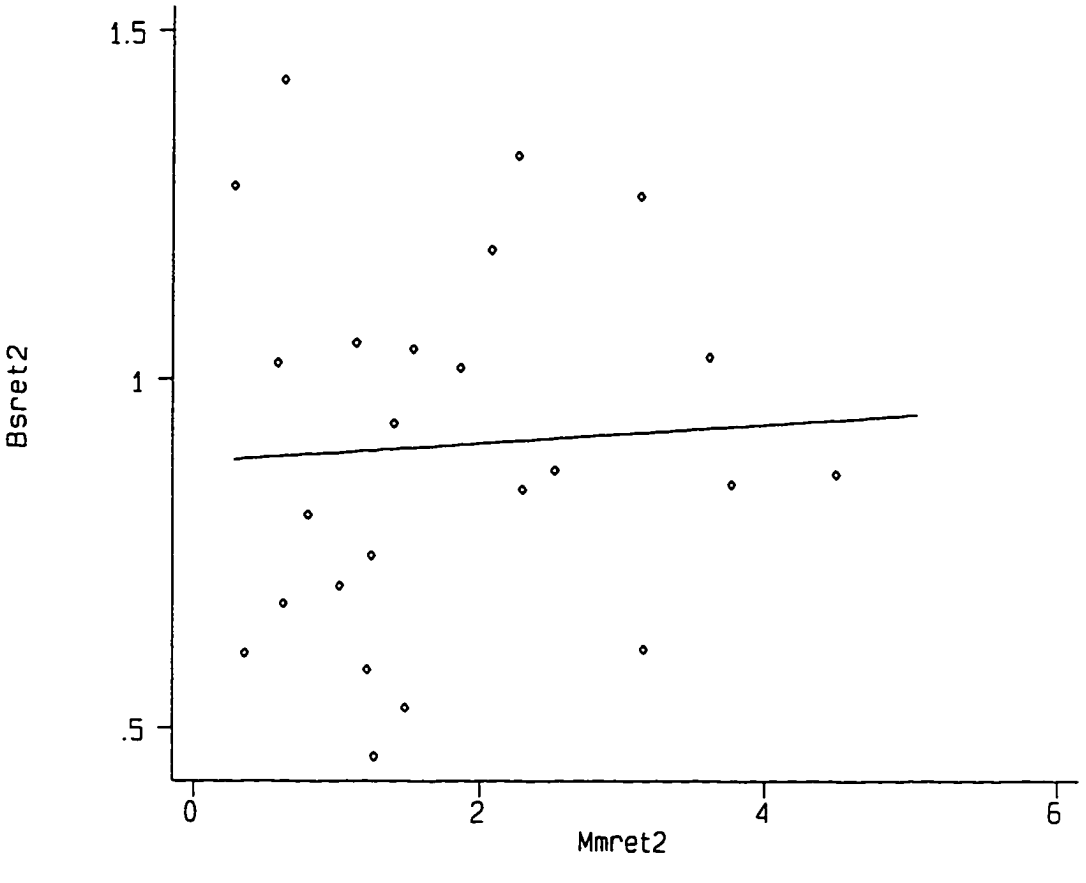


Figure 3: Correlation between maternal baseline breast milk retinol (µmol/L) and infants' baseline serum retinol levels (µmol/L) (n= 24); Mmret2= Maternal breast milk retinol levels and Bsret2 = Infants' serum retinol levels at baseline.

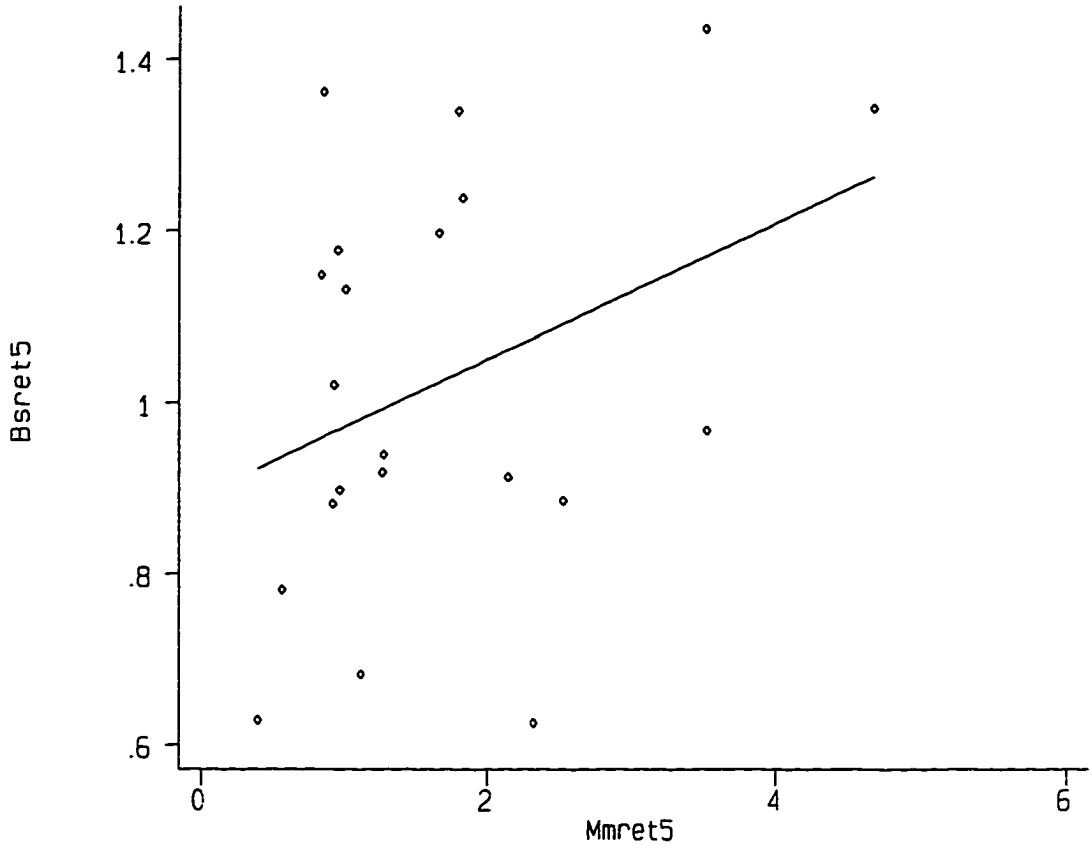


Figure 4: Correlation between maternal breast milk retinol (µmol/L) and infants' serum retinol levels (µmol/L) after supplementation (n=21); Mmret5 = Maternal breast milk retinol levels and Bsret5= Infants' serum retinol levels after β-carotene supplementation.

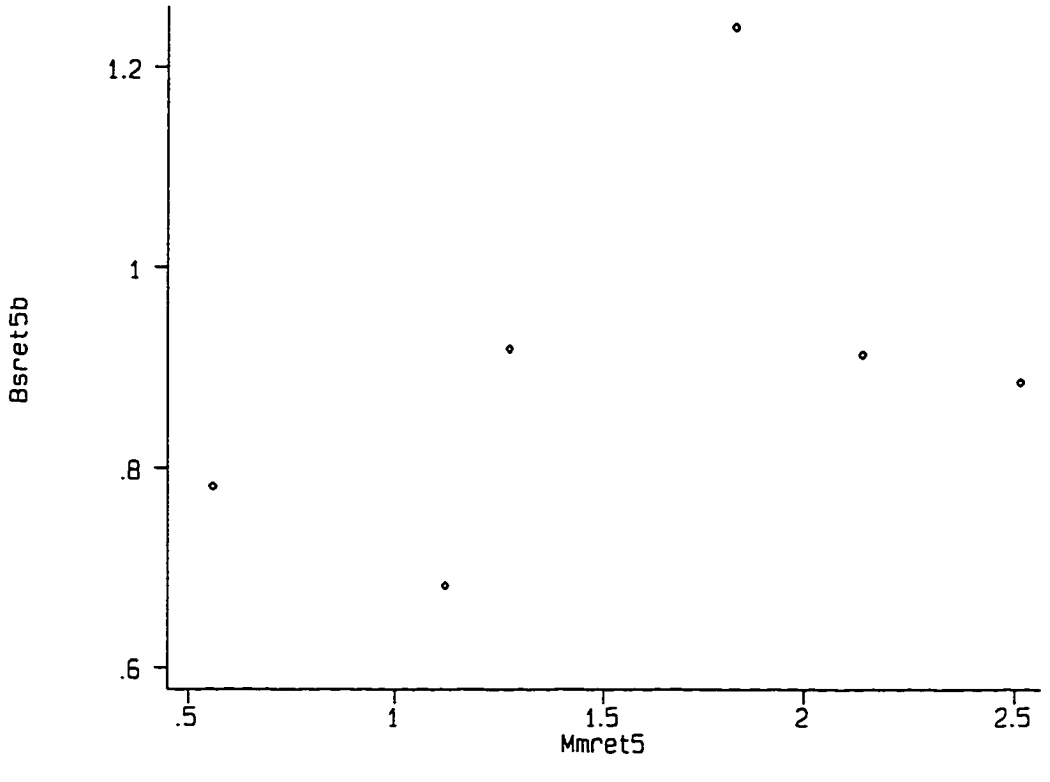


Figure 5: Correlation between maternal breast milk retinol (µmol/L) and the retinol levels (µmol/L) of infants of ≤4 months old after supplementation (r=0.43); Mmret5 =Maternal breast milk retinol levels and Bsret5b= Infants' serum retinol levels after β-carotene supplementation.

APPENDIX A-1

SAMPLE COLLECTION

SCHEDULE/PROTOCOL FOR BLOOD AND BREAST MILK COLLECTION IN THE STUDY FIELD IN HONDURAS

Thirty mg of pure β-carotene (Hoffmann-La Roche) was supplemented to 45 Honduran lactating women for 3 alternate days (a total of 90 mg of β-carotene) in a week long trial, and samples were obtained in the following ways:

First Sample collection (Day-1/Draw 1):

(Baseline)

Five ml of whole blood was collected from the lactating Honduran mothers before 30 mg of pure β-carotene supplementation.

Second Sample collection (Day-1/Draw 2):

(After first dose of supplementation)

Five ml of whole blood and about 10 ml of breast milk samples were collected from the mothers 5 hours after the first dose of supplementation with β-carotene. Two ml of infant's blood was also collected during this time.

Third Sample collection (Day-2/Draw 3):

After 24 hrs. of first supplement, 5 ml of whole blood was collected from the mothers.

Fourth Sample collection (Day-3/Draw 4):

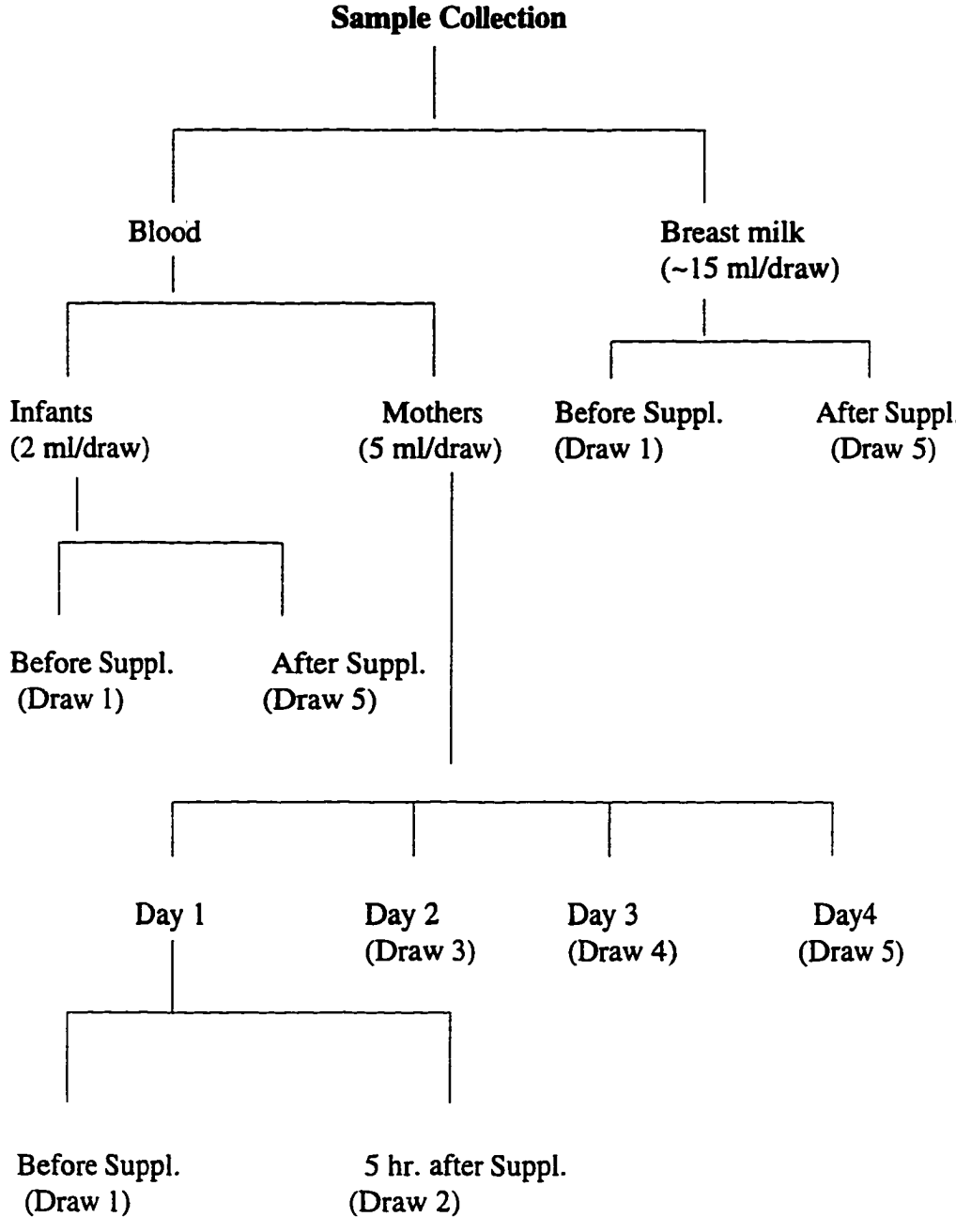
Before the 3rd supplement, 5 ml of blood was collected again from the mothers.

Fifth Sample collection (Day-4/Draw 5):

Both mothers' and infants' whole blood and mothers' breast milk samples were collected on 4th day of supplementation (last day of supplementation).

APPENDIX A-2

SAMPLE COLLECTION: FLOW CHART



APPENDIX B

BREAST MILK EXTRACTION PROCEDURE

EXTRACTION OF CAROTENOIDS AND RETINOL FROM BREAST MILK SAMPLES FOR HPLC ANALYSIS

Frozen mature breast milk samples were thawed at 37° C in a shaking water bath at 60 oscillations/min., to each 20 ml scintillation vial containing 4 ml thawed milk, 5 ml of absolute ethanol (Quantum Chemical, Tuscola, IL) and 3 ml of 50% (w/w) KOH were added. Samples were flushed with argon, capped with Teflon-lined phenolic caps (Titeseal, VWR, San Francisco, CA) and sonicated for 5 min. in a waterbath sonicator.

For the analysis of retinol, β-carotene, the samples were saponified for 16 hours at 25 ° C, at 130 oscillations/min. in an orbital shaker (New Brunswick Scientific, New Brunswick, NJ) in the dark. Following saponification, 4 ml of hexane was added to each sample. Samples were vigorously mixed by vortexing and sonicating for 5 min., then transferred to the 16 x 125 mm disposable culture tubes and centrifuged for 10 min. at 600 x g. The resulting hexane layer is retained and the aqueous layer re-extracted with 2 ml of hexane as before. The combined hexane layers were evaporated to 1-2 ml under nitrogen.

To remove water soluble impurities (e.g., KOH, polar lipids) from the combined hexane layer, samples were extracted with 2.5 ml of absolute ethanol and 3.5 ml of deionized water. This mmixture was vortexed and sonicated for 5 min. followed by centrifugation for 10 min. at 600 x g. The resulting hexane layer was removed and saved in a 1.5 ml microcentrifuge tube.

The aqueous layer was re-extracted with 1 ml of hexane as before. Then the combined hexane layers were evaporated to complete dryness with nitrogen and re-dissolved in 200 µl of THF-20% : methanol-80% mixture. Following 30-second sonication, 200 µl of the prepared sample was injected into the HPLC.

APPENDIX C

SERUM EXTRACTION PROCEDURE

EXTRACTION OF CAROTENOIDS AND RETINOL FROM SERUM SAMPLES FOR HPLC ANALYSIS

Reagents/Solvents:

1. **Methanol:** Pure; % of H_2O (water) is 0.018 ; UV cutoff is 204 nM.
2. **THF** (Tetrahydrofuran UV): Pure; % of H_2O is 0.012; UV cutoff is 211 nM
3. **Hexane UV:** % of H_2O is 0.002; UV cutoff is < 190 nm.
4. **BHT** (butyrated hydroxytoluene)
5. **Reconstitute solvent:** A mixture of 20% of THF, 80% of MeOH and 0.25 g/L of BHT.

Procedure in short:

Step-1: 250 µl serum

250 µl absolute ethanol (0.25 g/L BHT)

Vortex and allow to de-proteinized for 15 min.

Step-2: 1 ml hexane

Vortex and Sonicate for 2 min.

Spin (centrifuge) at 14,000 rpm for 2 min.

Step-3: Remove Hexane layer into a new (clean) microfuge tube

Dry under Nitrogen.

Step-4: Add additional 1 ml Hexane to serum tube

Vortex, Sonicate for 2 min.

Spin (centrifuge) at 14,000 rpm for 2 min.

Remove Hexane layer and combine with previous hexane residue drying under Nitrogen.

Step-5: After drying, reconstitute by:

Adding 250 µl of 20% THF/ 80% MeOH with 0.25 g/L BHT.

Sonicate 30 sec. *Spin in high speed for* 1 min. Either transfer it to the Autosampler or draw into a syringe to inject it into HPLC.

Step-6: Chromatography by HPLC.

APPENDIX D

RAW DATA

Data on characteristics of the normal Honduran lactating women.

ID #	age♣	preg♦	gestage✠	bwt♥	freq.-bfd¥	bsex♠	bage*
1	33	4	38	2.273	4	m	9
2	20	2	44	3.182	7	m	19
3	19	1	38	3.182	8	m	1
4	20	2	.	4.5	3	f	9
5	36	6	42	4.545	8	f	8
6	24	3	.	3.636	8	f	7
7	17	2	.	2.4	7	f	4
8	30	4	38	3.182	10	m	3
9	20	2	42	0	5	m	19
10	15	1	.	2.84	5	m	2
11	21	2	42	4.091	8	m	2
12	19	1	39	2.955	7	m	5
13	18	2	42	3.409	8	m	3
14	19	2	40	3.545	6	m	2
15	20	3	.	3.636	10	m	0.4
16	20	1	43	3.409	10	f	11
17	29	9	39	.	3	f	14
18	28	5	.	2.727	4	f	2
19	24	3	41	3.409	10	f	4
20	19	1	.	3.182	6	f	24
21	19	2	.	2.727	4	m	17
22	19	1	.	2.125	8	f	3
23	30	7	42	3.636	3	m	15
24	16	1	.	2.5	7	f	2
25	20	1	.	4.091	5	m	18
26	29	5	42	3.864	14	m	15
27	20	3	41	2.727	10	m	11
28	26	2	.	2.727	6	f	24
29	27	4	44	2.955	7	f	7
30	.	.	.	.	.	.	.

♣Maternal age in years; ♦number of pregnancies; ✠gestational age; ♥infants' birth weight in kilograms; ¥frequency of breastfeeding; ♠infants' sex; *infants' age in months.

Data on characteristics of the normal Honduran lactating women (continued).

ID #	age♣	preg♦	gestage✠	bwt♥	freq.-bfd¥	bsex♠	bage*
31	26	4	40	3.409	6	m	5
32	22	2	43	3.182	15	f	10
33	32	4	40	3.182	5	f	17
34	33	7	40	3.545	4	f	12
35	18	2	41	3.636	8	f	6
36	35	6	40	3.182	4	m	4
37	27	5	42	3.636	3	f	15
38	22	3	.	3	10	f	19
39	24	3	38	3.182	15	f	7
40	29	4	42	2.955	5	f	22
41	21	4	.	2.955	4	f	7
42	18	2	40	0	6	f	15
43	17	1	42	4.318	15	m	9
44	21	3	40	2.727	5	m	16
45	30	5	44	2.955	8	m	2

♣Maternal age in years; ♦number of pregnancies; ✠gestational age; ♥infants' birth weight in kilograms; ¥frequency of breastfeeding; ♠infants' sex; *infants' age in months.

Data on maternal serum β-carotene levels before and after supplementation.

ID #	Msbcar1	Msbcar2	Msbcar3	Msbcar4	Msbcar5
1	0.1087	0.16	1.0796	1.2472	1.2716
2	0.1873	0.2516	1.2838	1.0799	1.2013
3	0.2466	0.3026	1.3325	1.2716	1.1864
4	0.1161	0.1799	0.9024	1.0457	1.4065
5	0.0223	0.0923	0.8797	0.9554	0.995
6	0.0582	0.0617	0.7613	0.5968	0.6789
7	0.0961	0.1013	1.2312	0.9538	0.8152
8	0.1439	0.1734	1.1439	0.8588	1.4491
9	0.0592	0.0685	0.9409	0.7256	1.1853
10	0.1227	0.1134	1.2741	1.2597	1.0528
11	0.0601	0.0648	1.3327	0.6707	1.7345
12	0.1387	0.1115	1.0662	1.0206	1.014
13	0.2137	0.3029	1.9301	1.7164	1.6477
14	0.082	0.0841	0.6887	0.6711	0.5849
15	0.0467	0.0884	0.7459	0.5526	0.8751
16	0.0326	0.0485	0.7856	0.4419	0.7243
17	0.0433	0.0461	0.4511	0.5061	0.4544
18	0.041	0.0311	0.1	0.2412	0.1996
19	0.075	0.1067	1.7204	1.5167	2.4399
20	0.0444	0.079	0.5133	0.6265	0.6032
21	0.0951	0.1021	0.5605	0.7176	0.6241
22	0.0993	0.1613	0.8286	0.7086	0.6817
23	0.0819	0.196	0.5095	0.8073	0.8685
24	0.0656	0.1658	1.4381	2.1868	2.335
25	0.0919	0.1246	0.9181	0.345	0.4286
26	0.2315	0.175	1.0937	1.4287	1.3886
27	0.0339	0.071	0.3321	0.5184	0.576
28	0.0766	0.0852	0.3956	0.5861	0.589
29	0.1371	0.1657	0.6669	0.7003	.
30	.	.	.	.	.

Data on maternal serum β-carotene levels before and after supplementation (continued).

ID #	Msbcar1	Msbcar2	Msbcar3	Msbcar4	Msbcar5
31	0.1005	0.1244	0.8282	0.7487	0.8401
32	0.056	0.0818	0.36	0.6733	0.7326
33	0.0835	0.0578	0.9718	0.628	0.62
34	0.0803	0.1137	0.9107	0.8768	1.1196
35	0.1561	0.0831	1.0542	1.2934	1.6281
36	0.0641	0.0896	0.595	1.2199	1.3049
37	0.0691	0.0705	.	.	.
38	0.0345	0.0235	0.5164	0.9945	1.2416
39	0.0918	0.1039	0.6242	0.7224	0.55
40	0.0785	.	0.3329	0.7375	0.6793
41	0.0732	0.1067	1.4258	1.3131	1.518
42	0.0531	0.0314	0.4124	0.5795	0.7134
43	0.0881	0.096	1.2803	0.7774	1.0423
44	0.0744	0.0451	0.9724	0.7477	0.829
45	0.0579	0.0884	0.5708	0.6217	0.6999

Data on maternal serum retinol levels before and after supplementation.

ID #	Msret1	Msret2	Msret3	Msret4	Msret5
1	2.027	1.9677	1.9156	2.3606	2.1767
2	2.1105	2.1995	2.4135	1.9544	2.1724
3	1.8199	1.9106	1.7795	2.0176	1.8353
4	2.1542	2.0991	2.1193	2.2191	2.1772
5	1.1113	1.6258	1.4268	1.4506	1.5717
6	1.2884	1.2432	1.3808	1.3713	1.3687
7	1.0368	1.0414	1.1408	1.018	0.7582
8	1.6052	1.7648	1.9833	1.6841	1.7455
9	1.855	1.7068	2.0041	1.7059	1.7512
10	1.3773	1.2964	1.5093	1.2251	1.3255
11	2.5699	2.2162	2.2987	2.0535	2.1963
12	2.209	2.3485	2.3202	2.243	2.3093
13	2.1651	2.2749	2.0109	2.118	2.1278
14	1.2593	1.2203	1.2836	1.291	1.2731
15	1.2232	1.2505	1.0267	1.1458	1.2035
16	1.3643	1.3733	1.4396	1.2957	1.4322
17	0.8829	0.8536	1.0217	1.2839	1.1867
18	1.8424	1.7445	1.5436	2.1627	2.0671
19	1.8111	1.8557	1.7109	2.098	2.181
20	0.9847	1.0226	1.0256	1.0054	0.9267
21	1.7373	1.7301	1.7148	1.7202	1.7608
22	1.767	1.7313	1.6841	1.6016	1.7057
23	1.4937	1.5889	1.2825	1.4305	1.5548
24	1.6457	1.7703	1.4579	1.9432	2.144
25	1.4337	1.4952	1.465	1.2228	1.2024
26	1.6504	1.6064	1.7861	1.9161	1.7728
27	1.3709	1.5946	1.5905	1.6178	1.74
28	1.5982	1.5735	1.5197	1.5559	1.5725
29	1.5311	1.5735	1.5781	1.4743	.
30	.	.	.	.	.
31	1.9226	2.0362	1.9699	1.8667	1.8804

Data on maternal serum retinol levels before and after supplementation (continued).

ID #	Msret1	Msret2	Msret3	Msret4	Msret5
32	2.0462	2.4172	1.8257	2.1723	2.4115
33	1.2218	1.2463	1.5345	1.344	1.2794
34	1.1617	1.302	1.1363	1.2511	1.3017
35	1.9125	1.6335	1.6408	1.9006	1.9898
36	1.715	1.8935	1.438	1.9549	1.8533
37	1.7025	1.2853	.	.	.
38	1.203	1.1105	1.0649	1.3484	1.4959
39	0.9883	1.1089	0.8959	1.028	0.9961
40	1.4904	.	0.8877	1.7164	1.664
41	1.6601	1.7504	1.7357	1.7205	1.8377
42	1.4283	1.3058	1.3336	1.414	1.4189
43	1.4856	1.3716	1.4321	1.3179	1.5224
44	1.4236	1.3686	1.9496	1.6505	1.6484
45	1.123	1.1933	1.4119	1.4521	1.4407

Data on maternal breast milk β-carotene and retinol levels before and after supplementation.

ID #	Mmbcar2	Mmret2	Mmbcar5	Mmret5
1	0.0112	1.092	0.0235	0.7779
2	0.0199	1.8614	0.0847	1.663
3	0.0297	5.0417	0.1147	2.3765
4	0.0256	1.2403	0.0974	1.2783
5	.	2.254	0.0448	0.8633
6	0.0186	3.1213	0.1036	2.3056
7	.	.	.	.
8	0.0511	4.4915	0.5155	2.5105
9	.	3.754	.	.
10	0.0176	2.0296	0.1573	2.1187
11	0.0203	2.103	0.0794	1.5391
12	0.0173	1.3552	0.104	1.2522
13	0.0264	2.5036	0.0999	1.2711
14	0.0222	2.2857	0.0351	2.1377
15	0.0118	1.8036	0.0585	1.1911
16	0.0056	1.1386	0.0525	0.8456
17	.	.	.	.
18	.	.	.	.
19	0.011	1.2623	0.0603	0.5581
20	0.0051	0.6316	0.0276	0.3857
21	0.0097	0.5901		
22	0	3.5919	0.0407	1.8298
23	0.0163	2.3253	0.0884	1.9222
24	0.0298	1.3753	0.1866	2.7076
25	0.0178	1.2263	0.0873	1.4169
26	.	.	.	.
27	.	.	.	.
28	.	.	.	.
29	0.0178	0.8023	0.0659	0.9729
30				
31	0.0139	0.6349	0.2024	3.5023

Data on maternal breast milk β-carotene and retinol levels before and after supplementation (continued).

ID #	Mmbcar2	Mmret2	Mmbcar5	Mmret5
32	0.0138	1.48	0.1363	3.5108
33	.	.		
34	0.0256	2.0705	0.1296	1.7993
35	0.0016	0.277	0.0426	0.9633
36	0.0158	1.0249	0.0528	1.1205
37	0.0068	0.6688	.	.
38	.	.	.	.
39	0.033	1.8524	.	.
40	0.0154	1.5334	0.0872	1.0134
41	0.0076	1.2123	0.0538	0.928
42	0.0155	1.3992	0.038	0.9382
43	.	3.0941	0.1383	4.6887
44	0.0045	0.3525	0.1002	1.9893
45	0.0059	0.46	0.0678	1.7543

Data on infants' serum β-carotene and retinol levels before and after supplementation.

ID #	Bsbcar2	Bsret2	Bsbcar5	Bsret5
1	.	.	.	.
2	0.0991	1.0166	0.0624	1.1967
3	.	.	.	.
4	0.0319	0.7467	0.0558	0.9394
5	0.0793	1.3203	0.0584	1.3611
6	.	0.6123	0.0203	0.6253
7	.	0.7331	0	0.7552
8	0.0288	0.8632	0.0741	0.8848
9	0.031	0.8486	0.0389	1.3261
10	.	.	.	.
11	.	.	.	.
12	.	.	.	.
13	0.07	0.8686	0.0713	0.9182
14	0.1048	0.8414	0.0911	0.9124
15	.	.	.	.
16	.	1.0533	.	1.148
17	0.02	0.8751	0.0227	0.8319
18	0.0199	0.6842	0.03	0.9456
19	0.0304	0.4596	0.0469	0.7818
20	0	0.6775	0.0159	0.6293
21	0.0588	1.0236	0.054	0.9543
22	0.1066	1.0312	0.1522	1.2384
23	.	.	.	.
24	.	.	.	.
25	.	.	.	.
26	0.04	0.5911	0.0786	0.9707
27	0.018	0.5241	0.053	0.6737
28	0.0653	0.8977	0.0543	0.7041
29	0.0763	0.8049	0.0625	0.8976
30	.	.	.	.

Data on infants' serum β-carotene and retinol levels before and after supplementation (continued).

ID #	Bsbcar2	Bsret2	Bsbcar5	Bsret5
31	0.1374	1.429	0.1347	1.4335
32	0	0.5288	0.0105	0.9675
33	0.0421	1.406	0.0377	1.472
34	0.0367	1.1847	0.0418	1.3389
35	0.064	1.2779	0.0562	1.1772
36	0.1099	0.7034	0.0752	0.6817
37	.	.	.	.
38	.	0.9809	0.0239	1.3601
39	.	.	.	.
40	0.0827	1.0445	0.0836	1.131
41	0.0138	0.5831	0.035	0.8813
42	0.0242	0.9378	0.0264	1.0205
43	0.0716	1.2612	0.0526	1.3411
44	0.0168	0.6068	.	.
45	.	.	.	.

APPENDIX E

ABBREVIATION USED

Msbcar1	= Mother's 1st serum β-carotene sample	(Draw 1)
Msbcar2	= Mother's 2nd serum β-carotene sample	(Draw 2)
Msbcar3	= Mother's 3rd serum β-carotene sample	(Draw 3)
Msbacar4	= Mother's 4th serum β-carotene sample	(Draw 4)
Msbcar5	= Mother's 5th serum β-carotene sample	(Draw 5)
Msret1	= Mother's 1st serum retinol sample	(Draw 1)
Msret2	= Mother's 2nd serum retinol sample	(Draw 2)
Msret3	= Mother's 3rd serum retinol sample	(Draw 3)
Msret4	= Mother's 4th serum retinol sample	(Draw 4)
Msret5	= Mother's 5th serum retinol sample	(Draw 5)
Mmbcar2	= Mother's 1st breast milk β-carotene sample	(Draw 1)
Mmbcar5	= Mother's 5th breast milk β-carotene sample	(Draw 2)
Mmret2	= Mother's 1st breast milk retinol sample	(Draw 1)
Mmret5	= Mother's 5th breast milk retinol sample	(Draw 2)
Bsbcar2	= Infant's serum β-carotene post supplement	(Draw 1)
Bsbcar5	= Infant's serum β-carotene post-supplement	(Draw 2)
bage	= Infant's age at birth.	
bsex	= Infant's sex.	
bwt	= Infant's weight at birth.	
age	= Mother's age.	
preg	= Mother's no. of pregnancy.	

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