tachment 1

ACE SHEET) IDWP ETHICAL REVIEW COMMITTEE, ICDDR,B. Date January 4, 1994

incipal Investigator Amy Rice

Trainee Investigator (if any)

plication No. 94-001

Supporting Agency (if Non-ICDDR,B)

tle of Study The Effect of Retinol & ta-carotene supplementation in lactating men on breastmilk quality and vitamin A ~~atus in infants~~

Project status:
(X) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

rcle the appropriate answer to each of the following (If Not Applicable write NA).

Source of Population:
(a) Ill subjects — Yes (No)
(b) Non-ill subjects — (Yes) No
(c) Minors or persons under guardianship — (Yes) No

Does the study involve:
(a) Physical risks to the subjects — (Yes) No
(b) Social Risks — Yes (No)
(c) Psychological risks to subjects — Yes (No)
(d) Discomfort to subjects — (Yes) No
(e) Invasion of privacy — Yes (No)
(f) Disclosure of information damaging to subject or others — Yes (No)

Does the study involve:
(a) Use of records, (hospital, medical, death, birth or other) — (Yes) No
(b) Use of fetal tissue or abortus — Yes (No)
(c) Use of organs or body fluids — (Yes) No

Are subjects clearly informed about:
(a) Nature and purposes of study — (Yes) No
(b) Procedures to be followed including alternatives used — (Yes) No
(c) Physical risks — (Yes) No
(d) Sensitive questions — (Yes) No
(e) Benefits to be derived — (Yes) No NA
(f) Right to refuse to participate or to withdraw from study — (Yes) No
(g) Confidential handling of data — (Yes) No
(h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — Yes (No)

5. Will signed consent form be required:
(a) From subjects — (Yes) No
(b) From parent or guardian (if subjects are minors) — (Yes) No
6. Will precautions be taken to protect anonymity of subjects — (Yes) No
7. Check documents being submitted herewith to Committee:

___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
X Protocol (Required)
___ Abstract Summary (Required)
X Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
X Informed consent form for subjects
X Informed consent form for parent or guardian
___ Procedure for maintaining confidentiality
X Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

ree to obtain approval of the Ethical Review Committee for any changes lving the rights and welfare of subjects before making such change.

Amy Rice
Principal Investigator

Trainee

A-032054

Principal Investigator: Amy Rice

Co-Investigators: Andres de Francisco
Jyotsnamoy Chakraborty

Title: The Effect of Retinol and ß-carotene Supplementation in Lactating Women on Breastmilk Quality and Vitamin A Status in Infants

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Community Health Division, ICDDR,B

Protocol approved by: 3.1.94
Signature, Program Head / Date

Abstract Summary

Background: Vitamin A deficiency is public health problem which affects millions of preschool age children in Bangladesh and other developing countries. Current intervention programs do not reach breastfeeding infants. Improving the vitamin A intake of infants will potentially lower their risk of mortality during the later part of infancy and protect them from developing vitamin A deficiency during the high risk preschool age years.

The most important source of vitamin A for very young infants is the vitamin A they receive from breastmilk. Improving maternal vitamin A status will potentially result in an increase in breastmilk vitamin A levels and thus lead to an improvement in infant vitamin A status.

Objectives: The objectives of this study are to investigate the efficacy of maternal retinol supplementation (using a single 200,000 IU dose as currently recommended by the World Health Organization) and daily, dietary level β-carotene supplementation (used as a proxy for a dietary intervention) for increasing the vitamin A content of breastmilk and improving the vitamin A status of lactating women and their infants. To achieve this, a community-based, double-blind, individually randomized trial will be conducted among lactating women in Matlab, Bangladesh.

Significance: Studies examining the efficacy, and ultimately the effectiveness of these specific interventions have not been conducted. If both maternal retinol and β-carotene supplementation are shown to be efficacious, program planners may consider either one or both of these strategies when planning appropriate and effective interventions in the future.

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AIMS OF THE STUDY

General aim:

We propose to conduct a community-based, placebo-controlled, double-masked, individually randomized trial to investigate the efficacy of maternal vitamin A supplementation among an at risk population.

Specific aims:

- To establish the efficacy of daily, dietary level β-carotene supplementation to lactating women (as a proxy for dietary interventions) for increasing the vitamin A content of breastmilk and improving the vitamin A status of lactating women and their infants.
- To confirm the efficacy of maternal retinol supplementation with a single dose of 200,000 IU retinol (as currently recommended by WHO) for increasing the vitamin A content of breastmilk and improving the vitamin A status of lactating women and their infants.

The outcome measures for this study include the vitamin A status of the mothers (measured by serum retinol, the modified relative dose response (MRDR) test and conjunctival impression cytology (CIC)), the vitamin A content of breastmilk and the vitamin A status of the infants (measured by serum retinol).

Significance:

The results of this study will provide information regarding the efficacy of these two approaches to maternal vitamin A supplementation. If both retinol and β-carotene supplementation strategies are shown to be efficacious, future program planners may consider either one or both of these interventions when planning appropriate strategies targeted at improving the vitamin A status of lactating women and their infants.

Although WHO currently recommends maternal retinol supplementation soon after delivery for lactating women living in areas of endemic vitamin A deficiency, the recent work by Stoltzfus et al. in Indonesia using a 300,000 IU dose[1] was the first well-controlled trial establishing empirical evidence in support of this recommendation. This trial will use a 200,000 IU dose and we anticipate that this study will confirm the efficacy of maternal supplementation. This may be an effective intervention in Bangladesh if women come in contact with health care providers at delivery. Vitamin A capsules could potentially be included in the safe delivery kits which are being distributed to pregnant women by primary health care workers.

Many program planners would like to advocate dietary interventions as an effective and potentially more sustainable strategy. A number of nutrition education and home gardening projects are currently underway in Bangladesh. However, very little work has been done to critically evaluate the efficacy of dietary interventions for improving the vitamin A status of populations. Information regarding the impact of dietary interventions on improving the vitamin A content of breastmilk and the subsequent vitamin A status of infants is nonexistent. This trial will estimate the impact that dietary interventions targeted at lactating women may expect to have and will begin to address these issues.

ETHICAL IMPLICATIONS

Women who reside in the MCH-FP intervention area and voluntarily participate in DSS and MCH-FP program activities will be eligible for this study. Areas of interest for this study include the dietary habits of lactating women, infant feeding practices, morbidity patterns in infants and women and the growth patterns of infants. As such, we will not be asking the study participants sensitive questions. Confidentiality of the data will be maintained by coding the questionairres with study identification numbers, maintating a seperate identification key and keeping all information stored in locked files.

This study also deals with breastmilk quality and vitamin A status in mothers and their infants. To evaluate breastmilk quality, breastmilk samples will be collected from the women during each of the four study visits. Women will be requested not to feed their infant from one breast for a period of two hours to ensure appropriate sampling for assessing the vitamin A content of breastmilk. However, the infants will be able to feed from the other breast ad libitum. Other than the women perhaps experiencing temporary discomfort during breastmilk collection, we do not anticipate that this will have an adverse effect on the women or their infants.

To evaluate vitamin A status blood samples will be collected from the women by venipuncture on two occasions and once from the infants by heel stick, the modified relative dose respones test will be conducted on the women twice and conjunctival impression cytology (CIC) samples will be collected from the women on four occasions. Other than experiencing temporary discomfort during blood and CIC sample collection, we do not anticipate that these procedures will have an adverse effect on the women or their infants.

Women and their infants will receive health exams during their study visits at the Matlab clinic. They will receive treatment for any health problems which are detected during these visits.

BACKGROUND AND JUSTIFICATION

Vitamin A deficiency has long been recognized as an important international public health problem. Each year an estimated one million children worldwide develop xerophthalmia and over 500,000 become blind as a result of severe vitamin A deficiency.[2,3] An additional 20 to 40 million more may suffer from mild vitamin A deficiency.[4,5] Despite the efforts of many public health programs around the world, vitamin A deficiency remains one of the leading international nutritional problems in the 1990's.

Vitamin A deficiency manifests itself as a spectrum of signs and symptoms. While severe deficiency is characterized by the appearance of easily detectable clinical signs and symptoms, subclinical deficiency is not. Many efforts in the past have focused on preventing childhood blindness in populations with a high prevalence of clinical signs of eye disease. The point at which subclinical deficiency in an individual or population results in adverse effects is currently unknown. However, recent trials concerning vitamin A supplemenation and its effects on mortality and morbidity suggest that subclinical deficiency may also have serious consequences for children.

While most of the studies concerning vitamin A supplementation and childhood

mortality have demonstrated a reduction in mortality,[6,7,8,9] the effects on morbidity are less clear. Several studies have shown an association between vitamin A deficiency and diarrhea,[10] vitamin A deficiency and acute respiratory illness[11,12] and vitamin A status and measles outcomes.[13,14,15,16] Other studies have shown no effect of vitamin A supplementation on the incidence and severity of diarrhea or respiratory infections[17,18] and one recent study found an increase in prevalence of diarrhea and symptoms of respiratory infections following vitamin A supplementation.[19] Despite the current controversy regarding the exact physiological mechanism, the benefits of supplementary vitamin A capsule distribution programs among preschool age children are much greater than simply preventing nutritional blindness. Other types of interventions which reduce the prevelance of clinical and subclinical deficiency in young children may also have such benefits.

Several factors put preschool age children in developing countries at high risk of developing both clinical and subclinical vitamin A deficiency. Under normal circumstances, placental transfer of retinol is very limited, with the result that all infants are born with low liver reserves of vitamin A and depend on the vitamin A in breastmilk to build up their stores during the first few months of life.[20] However, preterm and low birthweight babies tend to have even lower liver levels than their fullterm counterparts.[21] In many developing countries, a large percentage of infants are born either early and/or with low birthweight, which may create an early disadvantage for them in terms of vitamin A status.

The most important source of vitamin A for infants is breastmilk. While it has been estimated that breastmilk from well-nourished women contains enough vitamin A to provide infants with their requirements for the first 6 months of life, several studies indicate that the vitamin A content of breastmilk decreases with worsening maternal nutritional status.[22,23,24] Infants of poorly nourished women in developing countries may consume inadequate amounts of vitamin A from breastmilk early in life. The next most important source of vitamin A for infants is weaning foods which are typically foods high in carbohydrates, but low in micronutrients, including vitamin A. A low intake of vitamin A from breastmilk and weaning foods during infancy puts preschoolers in developing countries at risk of developing vitamin A deficiency.

A variety of approaches have been utilized around the world to address the problem of vitamin A deficiency, with varying amounts of success. These include national programs of vitamin A capsule distribution, food fortification programs, home gardening projects, nutrition education programs and social marketing of vitamin A rich foods. Considering that vitamin A deficiency remains a significant public health problem, it is important to continue to look for new strategies to address this problem.

Current interventions do not reach infants. Children become eligible for supplementary vitamin A capsule distribution programs only after the age of 6 months-1 year and coverage, even in countries which actively promote such a program, is far from universal. Improving the vitamin A status of infants may decrease their risk of mortality later in infancy and help protect them from developing vitamin A deficiency as they get older.

Infants could receive additional vitamin A in several ways. One way is by directly supplementing them with vitamin A. Adding vitamin A supplementation to the list of services provided at the Expanded Program on Immunization (EPI) visits which are recommended for infants at 6, 10 and 14 weeks of life is a programmatically attractive strategy. However, results from a study recently conducted in Matlab, Bangladesh, indicate

that there is cause for concern regarding the safety and potential toxicity of vitamin A administered to such young infants.[25]

Another way to increase the vitamin A intake of infants is by improving the vitamin A intake of weaning foods, either through nutrition education or through food fortification programs. Nutrition education messages targeted at children and lactating women are currently being promoted in Bangladesh, but very young infants will potentially benefit from increasing their vitamin A intake before they reach the age when they start receiving weaning foods.

An additional way to reach infants is by improving maternal vitamin A status. The most important source of vitamin A for very young infants is the vitamin A they receive from breastmilk which originates from maternal stores of vitamin A. Although the relationship between the vitamin A status of lactating women, breastmilk quality and infant vitamin A status is poorly understood, a number of supplementation studies among lactating women have shown a positive impact on maternal vitamin A status and the vitamin A content of breastmilk.[26,27,28,29,30] Several of those studies which looked at the subsequent vitamin A status in infants showed an improvement in infant vitamin A status.

The fact that the serum retinol levels of these lactating women rose in response to supplementation suggests that they were initially deficient. Although little work has been done to investigate the prevalence of vitamin A deficiency among women of childbearing age in developing countries, it is suspected to be more widespread than is currently documented. Chronically low dietary intakes in combination with the demands of closely spaced, multiple pregnancies and subsequent periods of lactation compromise the overall nutritional status of women[31] and there is reason to believe that their vitamin A status is compromised as well. Maternal vitamin A supplementation programs for lactating women will potentially benefit both the mothers and their infants.

Improving maternal vitamin A status may be accomplished through direct supplementation with vitamin A capsules, through dietary improvement or through food fortification programs. Evaluations of the sugar fortification programs in Central America and MSG fortification in Indonesia have shown a positive impact on vitamin A status in lactating women. However, in many settings it is difficult to identify an appropriate food vehicle for fortification. Consequently, food fortification programs are not a commonly used means of specifically targeting lactating women to improve their vitamin A status.

The potential of maternal vitamin A supplementation as an effective intervention for improving the vitamin A status of lactating women has been officially recognized in the past decade. Both the International Vitamin A Consultative Group (IVACG) in 1986[32] and WHO in 1988[33] have recommended that lactating women who live in areas where vitamin A deficiency is a public health problem be given one oral dose of vitamin A (200,000 IU) within the first postpartum month. If given within that time frame, the risk of teratogenic effects on a subsequent fetus is negligible, while both the mother and breastfeeding infant will benefit. However, the recommendation for the 200,000 IU dosage is based on very little empirical evidence and may not be the optimal means of achieving maternal supplementation.

A randomized, placebo-controlled clinical trial of maternal supplementation recently conducted by Stoltzfus et al. in Indonesia provides the strongest evidence in support of retinol capsule supplementation. In that study, six months after the administration of a single dose of 300,000 IU of retinol, the vitamin A status (measured as mean serum retinol levels and the

retinol content of breastmilk) of women in the treatment group was better than that of the women in the placebo group. In addition, the proportion of infants in the treatment group with low serum retinol levels was less than that in the placebo group six months after maternal supplementation, indicating a transfer of vitamin A from the mothers to their infants.

Dietary interventions are an alternative and potentially more sustainable approach which could be used to improve the vitamin A status of lactating women and their infants. In many developing countries where vitamin A deficiency is a problem the major dietary source of vitamin A comes from plant foods in the form of ß-carotene. Home gardening programs and social marketing campaigns promoting ß-carotene rich foods for young children are underway in a number of countries. Although it is generally assumed that increasing the dietary intake of ß-carotene rich foods in at risk populations should result in an improved vitamin A status in individuals, very few critical evaluations of dietary intervention strategies have been conducted. We are not aware of any study which has been conducted using biochemical indicators to evaluate the impact of dietary interventions targeted at lactating women.

CONCEPTUAL FRAMEWORK

At the present time, very little is known about the relationship between the vitamin A status of lactating women, breastmilk quality and infant vitamin A status, especially among populations in developing countries. In general, breastmilk from women in developing countries is lower in retinol content and higher than ß-carotene than breastmilk from women in developed countries.[34] These differences may be attributed to variations in dietary habits. While women in developing countries consume the majority of their vitamin A in the form of ß-carotene from fruits and vegetables and very little retinol from animal sources, the opposite is true of women in developed countries. The diets of women in developing countries are also generally lower in fat than those of developed countries. However, the effect that these differences have on the composition of their breastmilk has not been definitively established.

The physiology of retinol and ß-carotene metabolism and transfer from maternal serum to breastmilk in humans is not well understood. Animal studies concerning the biosynthesis and transfer of vitamin A indicate that the majority of vitamin A in milk is esterified retinol. Retinol originating from liver stores and derived from circulating retinol bound to retinol binding protein has been shown to be esterified in the mammary gland of rats, sheep and monkeys.[35,36,37,38] The same mechanism for the transfer of retinol from serum to breastmilk has been proposed for humans.

While the majority of vitamin A in human breastmilk is retinyl esters, some is in the form of ß-carotene. It is unclear how efficiently dietary ß-carotene is metabolized and transferred from serum to breastmilk. Is dietary ß-carotene transferred intact directly into breastmilk or is it first converted to retinol? Is it then stored as retinol or transferred to breastmilk? How well can infants utilize ß-carotene from breastmilk? What effects other than improving the vitamin A status of infants may an increased ß-carotene intake have on infants? We are not aware of any previous studies which have been designed to investigate these issues. While this study will not address all of these points, it will begin to fill in some of the gaps in knowledge regarding maternal ß-carotene intake, breastmilk quality and infant

vitamin A status.

The vitamin A status of an individual at one point in time is influenced by a number of past and present factors. The vitamin A status of a lactating woman will depend on the current demands for vitamin A (normal physiological functioning plus lactation) as well as available body stores and current dietary intake. The design of this study is based on the current understanding of vitamin A physiology and information will be collected, when possible, on the factors listed in Table 1 which are important determinants of vitamin A status.

Table 1. Factors influencing the vitamin A status of mothers and infants and vitamin A content of breastmilk

Maternal Vitamin A Status-->	Breastmilk Vitamin A Content-->	Infant Vitamin A Status
• Body stores - Age - Parity • Dietary Intake • Morbidity • Supplementation status	• Volume of milk • Fat content • Retinol concentration (per gram of fat) • ß-carotene concentration (per gram of fat)	• Body stores • Breastmilk intake • Additional dietary intake • Morbidity

COUNTRY BACKGROUND--BANGLADESH

Vitamin A deficiency is a significant public health problem throughout much of the developing world, including Bangladesh.[39] The Bangladesh National Nutritional Blindness Study of 1982-83 the estimated prevalences for nightblindness among rural and urban preschool age children were 3.6% and 2.8% respectively. All stages of xerophthalmia among the children in this study exceeded the levels set by the WHO as criteria above which a public health problem is considered to exist.[40]

Three national nutritional surveys conducted in Bangladesh have documented low levels of dietary intake of vitamin A in the population.[41,42,43] The most recent survey conducted in 1982 showed the average intake of vitamin A to be only 38% of the recommended daily intake (RDI) with only about 10% of families receiving adequate amounts of vitamin A. A dietary intake study conducted by Zeitlin et al. among lactating women in rural Bangladesh in 1986 showed an average intake of 72% of the RDI with approximately 50% of the women consuming only 18% of the basal RDI.[44]

In 1973 the government of Bangladesh initiated a country-wide program to distribute high dose vitamin A capsules to preschool age children twice a year during April/May and October/November. This supplementation program remains an ongoing effort to address the problem of vitamin A deficiency in preschool age children. Despite these activities and more recent nutrition education initiatives and home gardening projects, vitamin A deficiency remains an important public health problem in Bangladesh.[45]

Breastfeeding is the dominant mode of infant feeding in developing countries.

Although breastfeeding has declined all over the world in recent decades, many women in Bangladesh routinely breastfeed their children for one to two years.[46,47,48] The widespread practice of breastfeeding and prevalence of vitamin A deficiency make Bangladesh an appropriate site for a research study to evaluate the efficacy of maternal vitamin A supplementation.

DESIGN AND METHODS

Human subjects

The proposed study will be a collaborative research effort conducted under the existing Joint Memorandum of Understanding regarding research between the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) and Johns Hopkins University School of Hygiene and Public Health in Baltimore, Maryland, USA (JHU). The protocol for this study will be reviewed and approved by the Research Review and Ethical Review Committees at ICDDR,B and the Committee on Human Research at JHU.

The study will be conducted in the 70 village area currently served by the Maternal and Child Health Family Planning (MCH-FP) intervention project of the ICDDR,B. The MCH-FP project operates in Matlab, an area located approximately 55 km southeast of the capital city of Dhaka. Residents of Matlab who voluntarily participate in the ongoing community surveillance and the MCH-FP project will be eligible for this study.

Since the majority of individuals in Matlab are illiterate, verbal consent will be obtained from all individuals recruited to participate in this study. Confidentiality of the data will be maintained by coding the questionairres and biological specimens with study identification numbers, maintating a seperate identification key and keeping all information stored in locked files in either Matlab or Dhaka.

Activity/Data collection schedule

This study will be a double-masked, placebo-controlled, individually randomized, community based trial of vitamin A supplementation of lactating women. Women and their infants will be enrolled in the study 14 days (±7 days) after birth and followed until the infant is 9 months old. In addition to receiving their regular MCH-FP visits every two weeks, study participants will come to the Matlab clinic for a special study visit four times over the course of a year.

Data collected for this study will come from information abstracted from MCH-FP records, questionnaires administered to study participants, through anthropometric measurements or result from laboratory tests on biological specimens. Table 2 outlines the schedule of data collection activities.

Table 2. Activity/Data collection schedule

Age of infant	VISIT TYPE Matlab clinic visit	MCH-FP home visit every two weeks
14 days ± 7 days	• Informed consent • Randomization/enrollment • Baseline household demographic and WI data† • Breastmilk collection • Maternal serum sample collection* • Maternal CIC sample collection • Infant weight and length • Maternal dietary assessment • Maternal supplementation begins	• Infant and maternal morbidity† • Infant dietary assessment†
3 months ± 7 days	• Breastmilk sample collection • Maternal serum sample collection* • Maternal CIC sample collection • Infant weight and length • Maternal height, weight and mid-upper arm circumference • Maternal dietary assessment	
6 months ± 7 days	• Breastmilk sample collection • Maternal serum sample collection* • Maternal CIC sample collection • Infant serum sample collection • Infant weight and length • Maternal dietary assessment	
9 months ± 7 days	• Breastmilk sample collection • Maternal serum sample collection* • Maternal CIC sample collection • Infant weight and length • Maternal dietary assessment	

† Information to be abstracted from existing MCH-FP records

* Maternal serum/CIC samples will be collected at two out of the four points in time from any one individual as local investigators felt that four blood draws would represent an excessive burden on subjects

Recruitment of participants

All pregnant women who deliver live infants in the intervention area served by the Maternal Child Health-Family Planning (MCH-FP) project during the study recruitment period (~ 2 months) will be eligible to participate in this study. Pregnant women identified through the ongoing MCH-FP surveillance will be invited to participate in this supplementation study towards the end of their pregnancies. If agreeable, the woman will be recontacted within two weeks (± 1 week) after delivery, screened for eligibility and enrolled in the trial. Births will be detected by intensive surveillance of the 80 community health workers (CHWs) in the MCH-FP project area during the recruitment period. Women who are not breastfeeding their infants will be excluded from the trial.

Baseline household demographic and socio-economic data

As part of the ongoing MCH-FP project activities, a MCH-FP CHW visits each household in the project area twice a month to record vital events and collects information on the health and reproductive status of mothers, family planning practices, infant feeding practices and morbidity data on children under five years old. Baseline socio-economic and demographic information concerning the households of study participants will be abstracted from records maintained by the MCH-FP project. The baseline questionnaire will include a section regarding a maternal history of night blindness during the most recent and/or previous pregnancies.

Randomization

Prior to the beginning of the study, investigators at JHU will draw up the randomization schedule. Randomization will occur at the level of the mother-infant pair. Women will be assigned to one of three treatment groups (ß-carotene, retinol or placebo) and one of six follow-up schedules for blood draws (0 and 3 months, 0 and 6 months, 0 and 9 months, 3 and 6 months, 3 and 9 months or 6 and 9 months). Although it would be preferable to collect blood samples from all women at all four points in time, investigators in Matlab feel that this represents an excessive burden on the mothers and would increase the likelihood of loss to follow-up. Table 3 outlines the various randomization cells in the study.

Table 3: Randomization Cells

Treatment Group	Combination of months at which blood will be drawn from an individual mother in the trial					
	0 & 3	0 & 6	0 & 9	3 & 6	3 & 9	6 & 9
Retinol	1	2	3	4	5	6
ß-carotene	7	8	9	10	11	12
Placebo	13	14	15	16	17	18

The randomization schedule will employ uniform allocation and a block design with blocks of equal length.[49] Women-infant pairs in the trial will be sequentially assigned a unique

identification number drawn from a list starting with 001. To ensure masking, the key to the enrollment code will be kept in with investigators at Johns Hopkins University and broken only after the conclusion of the trial.

Study participants will not be matched on any baseline factors prior to randomization. The population in the MCH-FP area is reasonably homogeneous with respect to socio-economic status, age structure etc. and based on data from previous years we expect births to be relatively evenly distributed in the area. In light of the already complex randomization scheme, the marginal benefits of matching participants on any baseline factors are outweighed by the logistical difficulties involved in attempting to do so.

Supplementation schedule

Lactating women enrolled in the study will belong to one of three treatment groups: β-carotene, retinol or placebo. They will receive daily supplementation capsules over the course of nine months following the schedule outlined in Table 4. MCH-FP CHWs will deliver packets containing a two week supply of capsules to the study participants' homes during their regular fortnightly visit.

Table 4. Supplementation schedule

Treatment Group	Day of study											
	0	1	2	3	4	5	6	7	8	..	..	252
ß-carotene	B	B	B	B	B	B	B	B	B	B	B	B
Retinol	R	X	X	X	X	X	X	X	X	X	X	X
Placebo	X	X	X	X	X	X	X	X	X	X	X	X

B= 7,800 μg β-carotene in oil
R= 200,000 IU retinol palmitate
X= placebo

Compliance will be assessed by having the CHWs collect the used packets of capsules and count and record the number of remaining capsules each time a new supply is delivered. Spot checks of compliance with the daily dosing regime will be conducted on a subset of study participants by project specific field workers.

Supplementation capsules

Three types of supplementation capsules will be used in this study: β-carotene capsules containing 7,800 μg of β-carotene in oil, retinol capsules containing 200,000 IU of retinyl palmitate and placebo capsules containing neither retinol or β-carotene. The capsules will be manufactured by Banner/Pharmacaps (Elizabeth, NJ) to resemble each other in size and color as much as possible.

ß-carotene: Because it is difficult to document, much less control what people eat, daily ß-carotene capsules containing the US RDA (1300 retinol equivalents (RE)) of vitamin

A for lactating women will be administered during the nine months of the study. This will be used as a controlled way to simulate a dietary intervention which promotes ß-carotene rich foods and results in lactating women consuming the US RDA of ß-carotene every day. Using the conventional conversion factor of 1 RE equal to 6 μg ß-carotene, the daily dose of ß-carotene equals 7800 μg. This conversion factor is based on the assumption that 30% of the consumed ß-carotene is absorbed and that the ß-carotene is converted to retinol in a 1:1 ratio.

A number of factors may affect the validity of using ß-carotene capsules to simulate the effect of a dietary intervention. Some studies suggest that ß-carotene given in a capsule is absorbed better than that found in food.[50] While it is clear that some oil or fat is essential for the absorption of ß-carotene, the minimum required amount and the relationship between varying amounts of dietary fat and efficiency of absorption is not well described. Some, but not all studies, have shown a greater impact on the serum retinol levels of study subjects when ß-carotene supplements (as capsules or food) were consumed with additional oil, rather than the ß-carotene supplements alone.[51,52,53,54]

The ß-carotene capsules in this study will be manufactured by Banner/Pharmacaps in a manner similar to their regularly produced, over-the-counter daily ß-carotene supplement capsules sold on the American market. The ß-carotene will be dissolved in a small amount of oil, which should be sufficient to stimulate absorption.

The mechanism of the conversion of ß-carotene to retinol is currently an area of intense debate. Theoretically, one molecule of ß-carotene could yield 2 molecules of retinol, depending on the mechanism of conversion. However, some animal experiments show a conversion ratios ranging from less than 1:1 to 1:2, with an increasing conversion ratio as the dosage of ß-carotene decreased.[55] For ß-carotene intakes in the range of the daily US RDA the most widely accepted and used conversion ratio is 1:1.

The literature regarding ß-carotene metabolism and its interrelationship with other nutrients is limited. While a number of factors have been proposed to influence the metabolism of ß-carotene in humans including the fat content of the diet, zinc status, protein status and current vitamin A status, the literature regarding these factors is contradictory.[56] This study will be conducted among a population of lactating women who are presumed to be subclinically deficient. It is unknown how efficiently these women will convert the ß-carotene consumed in the supplementation capsules.

The bioavailability of ß-carotene present in foods is very difficult to determine. Studies show that the absorption and bioavailability of ß-carotene from foods varies widely, depending on the type of food, methods of preparation and other components of the diet.[57,58,59,60] It is difficult to accurately simulate something that varies so widely.

Given the lack of more definitive information and the even more difficult task of attempting to conduct an efficacy study of ß-carotene supplementation using food, daily capsules containing 7800 μg ß-carotene in oil will be used to simulate a dietary intervention and to investigate the efficacy of ß-carotene supplementation.

Retinol: The retinol supplementation capsules will contain 200,000 IU retinyl palmitate and 40 μg tocopherol. This is the dose currently recommended by the WHO for supplementation of lactating women. While this may not be the optimal dosage for maternal vitamin A supplementation, the aim of this study is to investigate the efficacy of the current WHO recommendation.

Placebo: The placebo capsules will not contain any retinol or ß-carotene. This group

is being included for comparison purposes.

Vitamin A status

The main outcomes of this study are the vitamin A content of breastmilk and the vitamin A status of the mothers and infants. Indicators of vitamin A status which are commonly used in research studies include serum retinol levels, the relative (and modified relative) dose response test, conjunctival impression cytology, dark adaptation and the determination of dietary intakes of vitamin A, none of which is ideal. Utilizing a combination of indicators rather than a single one provides a better estimate of the overall vitamin A status of a population. In this study serum retinol and ß-carotene levels will be measured on mothers and infants, the modified relative dose response test and conjunctival impression cytology will be conducted on women and information on dietary practices will be collected from both mothers and infants.

Serum retinol and ß-carotene levels: The most commonly used method of vitamin A assessment for population studies is the determination of serum retinol levels, a method which measures the amount of retinol circulating in the bloodstream of individuals. Due to homeostatic control, serum retinol levels reflect liver stores only at the extremes of very low and very high body stores. While the serum retinol level of an individual is not necessarily a good indicator their vitamin A status due to day to day variability, the mean serum retinol level of a population is a useful indicator of the group's status.[61]

In this study, blood samples will be collected from mothers at two points in time (either 0, 3, 6 or 9 months) by venipuncture. The serum samples will be collected and stored to avoid the degradation of retinol and ß-carotene. Precautions include protecting the samples from light and oxidation and keeping them appropriately frozen until analysis. Stability studies conducted with plasma samples showed a negligible loss of retinol and ß-carotene after 28 months with storage at -70° C or after 5 months at -20° C.[62] After collection and processing at the clinic in Matlab, the serum samples will be temporarily stored at -20° C until they can be transported to Dhaka, where will be stored at -70° C until they are analyzed for retinol and ß-carotene using high-performance liquid chromatography.[63]

Serum samples will be collected by heelprick from all of the infants once at the six month visit, stored and analyzed for their retinol and ß-carotene content in the same manner as maternal serum samples. The six month, rather than the nine month visit, was chosen in order to assess the impact maternal supplementation has on infant vitamin A status before the infants become eligible to receive supplemental vitamin A capsules at the age of six months. The government of Bangladesh recommends that all infants receive 100,000 IU of vitamin A at six months of age.[64] All infants in the study will receive supplementation according to these guidelines. They will continue to be followed for the next three months for anthropometric, dietary and morbidity assessment.

Conjunctival impression cytology (CIC): Conjunctival impression cytology is another technique under development as an indicator of vitamin A status of individuals.[65] This method measures histological changes in the conjunctiva of the eye which may occur as a result of vitamin A deficiency. The technique has been successfully used in field trials with children, but has been shown to be more problematic when used with infants or children less than three years old.[66] However, the method may have potential for use as an assessment tool in adults.[67] CIC samples will be collected only from the mothers during the clinic visits at

baseline, three, six and nine month clinic visits.

Modified relative dose response (MRDR) test: Recent work indicates that the modified relative dose response test is another useful indicator of marginal vitamin A status.[68,69] This method indirectly assesses liver stores by measuring the release of retinol binding protein (RBP), which accumulates in the liver during marginal and severe vitamin A deficiency, following the administration of a test dose of 3,4-didehydoretinyl acetate. This technique has been successful used in field studies with children, but not yet among a population of lactating women. In this study the MRDR test will be conducted in women, but not infants, during the same clinic visits when blood is drawn for the determination of serum retinol and ß-carotene.

Iron status

Although several studies suggest an association between vitamin A deficiency and poor iron status, the mechanism and public health importance of this interaction are unclear.[70,71,72] Iron deficiency is a common condition among women in the developing world, including Bangladesh. In a clinic based trial of 129 women of childbearing age in Matlab, 48% of the women surveyed had hemoglobin levels less than 11 g/dl and 20% had levels less than 10 g/dl.[73] Currently, all women in the MCH-FP area are offered iron-folate capsules every two weeks during home visits throughout their pregnancy and period of lactation. While compliance with iron-folate supplementation has not been documented in Matlab, it is suspected to be low. A recent evaluation of an iron supplementation program among pregnant women in Dhaka showed very poor compliance with a daily supplementation regime.[74]

In this study, delivery of iron-folate capsules to study participants' homes during pregnancy and lactation will be recorded. Hemoglobin measurements will be determined on blood samples from both the mothers and infants using a HemoCue hemoglobinometer (Helsingborg, Sweden). Associations between the vitamin A and iron status of both the woman and infants will be investigated during the analysis of the study.

Breastmilk vitamin A content

The retinol and ß-carotene present in breastmilk is associated with the fat portion of the milk.[75] The fat content of breastmilk has been shown to vary widely according to time of day, time since the last feed and whether the milk is fore or hind milk.[76] However, the retinol concentration per gram of fat in breastmilk has been shown to be more constant within an individual. Minimizing the variability in the fat content of breastmilk due to sampling procedures is the most important factor to consider in when attempting to reduce sampling induced variability in breastmilk vitamin A determinations.[77]

The following standardized breastmilk collection technique will be used in this study. Samples will be collected from women in the morning from a breast that has not been nursed for at least 2 hours. Manual breast pumps (White River, Laguna Hills, CA) will be used to completely express the milk from the one breast. After manual homogenization, the fat content of the samples will be determined using the creamatocrit method.[78] The remainder of the sample will be aliquoted and temporarily stored at -20° C until they can be transported to Dhaka for further analysis.

Since there is no data available regarding the stability of retinol and ß-carotene in

breastmilk and studies indicate that ß-carotene levels in plasma stored at -20° C begin to decline after 5 months of storage, the breastmilk samples will also be stored at -70° C in Dhaka until analysis of retinol and ß-carotene using HPLC.[79] Breastmilk will be collected from mothers at baseline, three, six and nine month clinic visits.

Maternal dietary assessment

Women will be consuming ß-carotene and retinol as part of their normal diets throughout this study, in addition to receiving β-carotene or retinol supplementation. However, since the treatment groups are assigned randomly, it is improbable that women in the various groups will consume different diets during the course of the study.

Twenty-four hour recall interviews are an appropriate method to use to measure current dietary intake in groups of subjects.[80] A twenty-four hour recall interview will be conducted with each study participant at the baseline, three, six and nine month clinic visit, with special emphasis being placed on foods containing ß-carotene, retinol and fat. The data will then be coded and food composition tables from Bangladesh will be used to estimate the dietary intake of vitamin A for each woman.[81] This method of dietary assessment will be sufficient to establish group comparability and describe the dietary practices of the study population.

Several studies in rural Bangladesh have documented the importance of seasonality in dietary intake of vitamin A.[82] Zeitlin et al. found ß-carotene rich vegetables (dark green leafy vegetables, pumpkin, green beans and chili peppers) and mangoes to be the most important sources of vitamin A, while retinol from animal products contributed negligible amounts to the total dietary intake of vitamin A in the women in their study.[39]

Although seasonal availability of fruits and vegetables will influence the amount of vitamin A in the women's diets and their vitamin A status during this study, seasonality should not affect the internal validity of this study. Recruitment will take place over a relatively short period of time (two months) and block randomization will be used to assign women to treatment groups, reducing the probabability that women in the different groups will have different dietary intakes of vitamin A during the study. The relationship between seasonal differences in dietary intake and women's vitamin A status is not a central question of this research and will not be addressed unless a more comprehensive dietary assessment methodology is utilized.

Infant dietary assessment

Breastmilk is expected to be the major source of vitamin A for in the infants in this study. In a study conducted in Matlab, Brown et al. found that over 80% of the vitamin A intake in children less than one year old came from breastmilk.[83] Basic data on infant feeding practices (exclusive breastfeeding, partial breastfeeding, no breastfeeding, supplemental foods) is routinely collected as part of the MCH-FP surveillance questionnaires and will be used in the analysis of this study. No attempt will be made to assess the amount of breastmilk fed to the infants. At the three, six and nine month visits a food frequency questionnaire will be used to record any types of supplemental foods the mother has given to the infant since the previous visit.

Anthropometric assessment

While vitamin A is necessary at the cellular level for differentiation and growth, data regarding the impact of vitamin A supplementation on growth in infants and children is limited. Since it is unlikely that gestational age or birth weight of the infants will be assessed, the baseline weights and lengths of infants will be used to examine group comparability. Weight and length measurements will be repeated at the 3, 6 and 9 month visits.

Mothers in the study will have weight, height and mid-upper arm circumference measurements taken only at the 3 month visit in order to examine the comparability of the treatment groups. This, rather than the enrollment visit, was chosen in order to reduce the variability found in measurements of weight in post-partum women soon after delivery. Body-mass index (BMI) will be calculated and used as an indicator of general nutritional status.

Morbidity assessment

Morbidity in the infants and mothers may adversely affect their vitamin A status. During the regular fortnightly visits conducted by the CHWs in the MCH-FP project mothers are asked to recall any episodes of pneumonia or diarrhea their child may have had during the past two weeks as well as to report any other episodes of morbidity their children or they may have experienced. This information will be used in the analysis of factors influencing the vitamin A status of mothers and infants.

SAMPLE SIZE

The outcome in this study with the most direct programmatic importance is the prevention of subclinical vitamin A deficiency in infants. Vitamin A supplementation of lactating women is expected to improve the vitamin A status of supplemented women, increase the vitamin A content of their breastmilk and change the distribution of serum retinol levels of their infants. Due to the mechanisms of retinol and β-carotene metabolism and storage, supplementation may produce a shift in the distribution of serum retinol values of the infants without producing a statistically significant change in the mean serum retinol value of the groups. For this reason, the sample size calculation is based on a examining the difference in the proportion of infants with low serum retinol values in the treatment groups. This is the outcome which will require the largest sample size.

Since no data exists on the serum retinol levels of a representative group of infants from Matlab, data from the maternal retinol supplementation trial conducted by Stoltzfus et al. in Indonesia[32] are used along with the limited data from Bangladesh to estimate the sample size for this trial. The Indonesian trial compared the effects of a one time maternal supplement of 300,000 IU retinol vs. a placebo treatment on the vitamin A status of infants. At the end of the study, the proportion of infants aged 6-7 months in the placebo group with low levels of serum retinol (defined as <15 μg/dl) was 36%, whereas only 15% of the infants in the retinol treatment group had low serum retinol levels.

Data on the serum retinol levels of infants in Bangladesh comes mainly from clinic based trials. In one dataset from the Matlab area, 39% of infants 0-5 months (n=98) had

serum retinol levels less than 20 μg/dl,[84] which is the cutoff level conventionally used to designate low serum retinol levels. Data on the serum retinol levels of 171 infants are available from trials conducted at the ICDDR,B during 1990-1992.[85] Of those studied, 127 were between the ages of 1-8 months with 80% having serum retinol levels less than 20 μg/dl. Of those infants 5-8 months old (n=19), 47% had serum retinol levels less than 20 μg/dl. These infants are in the age range most similar to the infants which will be examined at the end of this study.

In choosing the sample size for this trial, the proportion of infants with serum retinol levels less than 20 μg/dl in Matlab is assumed to be close to 50%. The desired detectable decrease in the proportion of infants with low serum retinol values between 1) the retinol and placebo groups or 2) the ß-carotene and placebo groups is 50%. The difference in the proportion of infants with low serum retinol levels between the retinol and ß-carotene groups is expected to be much smaller. This trial is designed to assess the efficacy of each of the treatments vs. a placebo, not to directly compare them to each other. The ability to draw conclusions about the comparative efficacy of the two interventions will depend on the magnitude of the observed differences. Finding that either one or both of the treatments is efficacious in reducing subclinical vitamin A deficiency in infants will be an important result.

The sample size required to detect a difference in proportions between 50% and 25% with an α level of 0.05 and 80 per cent power is 59 infants. The sample size will be increased to account for approximately 20% loss to follow-up for whatever reason, resulting in a final sample size of 216 mother-infant pairs. The study participants will be distributed among the randomization cells as shown in Table 5.

Table 5. Number of study participants (mother-infant pairs) by randomization cells

Treatment Group	Combination of months at which blood will be drawn from an individual mother in the trial						Total (by group)
	0 & 3	0 & 6	0 & 9	3 & 6	3 & 9	6 & 9	
Retinol	10[1] (12)[2]	10 (12)	10 (12)	10 (12)	10 (12)	10 (12)	60 (72)
ß-carotene	10 (12)	10 (12)	10 (12)	10 (12)	10 (12)	10 (12)	60 (72)
Placebo	10 (12)	10 (12)	10 (12)	10 (12)	10 (12)	10 (12)	60 (72)

[1]Number desired

[2]Number recruited after increase per cell for dropouts

N=180 (216)

The chosen sample size will result in sufficient power to detect the differences in proportions between groups shown in the shaded region of Table 6.

Table 6. Number of infants required per treatment group to detect a specified difference in proportions between two groups[§]

		Proportion in placebo group								
		.20	.25	.30	.35	.40	.45	.50	.55	.60
Proportion in ß-carotene or retinol group	.10	200 268	101 135	63 84	44 59	33 44	26 34	21 28	17 23	14 19
	.15	906 1212	251 336	122 163	74 98	50 67	37 49	28 38	22 30	18 24
	.20		1094 1464	294 394	139 186	82 110	55 74	40 53	30 40	24 31
	.25			1250 1674	329 441	153 205	89 119	59 79	42 56	31 42
	.30				1376 1842	357 478	163 219	94 126	61 82	43 58
	.35					1470 1968	376 504	170 228	97 130	63 84

[§]Upper figure power=80 % power
Lower figure power=90 % power
Two-sided significance test with α=0.05

The following formula was used for these calculations[86]

$$n = \frac{(z_\alpha + z_\beta)^2 2p(1-p)}{(p_1-p_2)^2}$$

p_1 = proportion in intervention group
p_2 = proportion in placebo group
p = average proportion

DATA MANAGEMENT

Data in this study will come from several sources 1) primary data collected directly from study participants 2) laboratory results from the analysis of blood and breastmilk samples and 3) MCH-FP records. The software program dSurvey (Helkorn Pty Ltd, Pearce Act, Australia) will be used to manage data entry and cleaning and to document the data collection process. Primary data collected from study participants and laboratory results will be directly entered into the study database using dSurvey. Data abstracted from MCH-FP records will be incorporated into the study database. Completed data entry forms, laboratory results and database diskettes will be stored in a locked cabinet either in the field station in Matlab or in Dhaka.

DATA ANALYSIS

Statistical analyses will be carried out using the Statistical Analysis System (SAS Institute, Cary, NC) program on either a personal or mainframe computer. The general data analysis plan is as follows:

Description of the baseline characteristics of the study population

* Examine the distribution of baseline characteristics of all participants initially eligible for the study (some background will be available on all individuals from MCH-FP records)
* Compare the characteristics of those who enrolled in the study with those who refused to participate
* Examine baseline comparability between treatment groups
* Compare the distribution of baseline characteristics between participants who completed the study vs. those who dropped out or were lost to follow up
* Look for differential dropout by treatment group or follow up schedule

Comparison of infant and maternal vitamin A status between treatment groups

* Compare the vitamin A status of mothers and infants within and between groups across time using the following variables
  - infant serum retinol levels at 6 months
  - infant serum ß-carotene levels at 6 months
  - maternal serum retinol levels at 0, 3, 6 and 9 months
  - maternal serum ß-carotene levels at 0, 3, 6 and 9 months
  - maternal CIC results at 0, 3, 6 and 9 months
  - maternal MRDR results at 0, 3, 6 and 9 months

Differences in serum retinol levels will be examined using means and proportions. Since standards for serum ß-carotene levels do not exist, the data will be examined for differences in means. CIC and MRDR test results will be classified as normal or abnormal according to standard definitions. The data for CIC and MRDR will be examined for differences in proportions.

Comparison of indicators of vitamin A status

* Examine the correlation between CIC, MRDR and serum retinol values for individual women in the study
* Calculate the sensitivity, specificity, positive and negative predictive value of CIC and MRDR for assessing poor vitamin A status (using serum retinol levels as the gold standard)

Comparison of breastmilk quality between treatment groups

* Compare breastmilk quality within and between groups across time using the following variables:
  - volume of samples collected
  - per cent fat in the samples collected
  - retinol level/gram fat
  - ß-carotene level/gram fat

The data will be examined for differences in means.

Consideration of potentially confounding factors

If the treatment groups are not comparable with regard to baseline characteristics or other factors measured during the course of the study, for example dietary intake of vitamin A, those differences may influence the outcome measures and confound the results. Stratification and multiple logistic regression will be used to control for the effects of confounding variables.

LIMITATIONS

When interpreting the results of this study, several limitations related to the design should be kept in mind. One major assumption is the validity of using β-carotene supplementation as a proxy for β-carotene intake from dietary sources. It is unknown how closely the supplement will approximate β-carotene intake from foods. The dosage (equivalent of 1 US RDA for lactating women) and timing of delivery (daily) of the ß-carotene supplements were chosen based on our current understanding of ß-carotene metabolism and will probably represent the maximum effect that could be achieved by a dietary intervention.

Due to logistic constraints, it will be impossible to assure complete compliance with the supplementation regime. However, the first dose will be taken under the supervision of project staff, which will ensure that all women in the retinol group receive the proper dose. Compliance with the supplementation regime will be estimated through spot checks by field staff during routine visits and by counting the capsules remaining in supplementation packets as they are exchanged for new packets. The amount of ß-carotene consumed by study participants will be estimated from compliance figures and utilized during data analysis.

The generalizability of the findings from this study will depend on the extent to which study participants are representative of women in other parts of Bangladesh, South Asia or the remaining developing world. While the magnitude of the effect that may result from retinol and β-carotene supplementation may differ in other settings, the direction of the effect is expected to be the same.

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Flow Chart of Activities

Activities	Months of Study																																			
	1993				1994												1995												1996									
	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J
Protocol development	X	X	X	X	X																																								
ICDDR,B approval process	X	X	X	X	X	X																																							
JHU approval process						X																																							
Forms development and pretesting				X	X	X	X	X																																					
Field staff recruitment and training							X	X																																					
Enrollment									X	X																																			
Follow-up									X	X	X	X	X	X	X	X	X	X	X																											
Data entry and cleaning									X	X	X	X	X	X	X	X	X	X	X	X	X	X																								
Biochemical analyses													X	X	X	X	X	X	X	X	X	X																								
Data analysis/report writing																													X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Specific Tasks for each Investigator

Principal Investigator:
Amy Rice

- Develop and finalize protocol
- Develop and finalize questionnaires
- Select field and laboratory staff
- Assist with training of field and laboratory staff
- Assist with supervision of Matlab field staff and Matlab and Dhaka laboratory staff
- Data analysis and interpretation, writing of findings and recommendations

Co-Investigator:
Dr. Andres de Francisco

- Assist with finalization of protocol and questionairres
- Assist with selection of field and laboratory staff
- Assist with supervision of Dhaka laboratory staff
- Assist with data analysis and interpretation, writing of findings and recommendations

Co-Investigator:
Jyotsnamoy Chakraborty

- Assist with selection of field and laboratory staff
- Assist with supervision of Matlab field staff
- Assist with the coordination of all logistical tasks in Matlab
- Assist with data analysis and interpretation, writing of findings and recommendations

EXHIBIT (B)

BUDGET

RETIBETA STUDY — AUG. 93

BUDGET

(IN US$)

3100 LOCAL SALARIES

DESIGNATION	LEVEL	POSITIONS	MAN-MONTHS	RATE/MONTH	AMOUNT
MANAGER H.S.	NOC	1	1	1100	1100
MEDICAL OFFICER	NOB	1	9	800	7200
TECHNICIAN DHAKA	GS5	1	12	500	6000
FIELD RES. OFF	GS5	1	4	400	1600
SHA	GS4	3	36	400	14400
DATA MANAG. OFF	GS4	1	1	400	400
DATA ENTRY CLERK	GS3	1	12	300	3600
RES. ASST. MATLAB	GS3	1	12	300	3600
CLINIC ATTENDANT	DW	1	12	50	600
CHW	CHW	*80 (15%)	14	100	16800
				SUBTOTAL	55300

3200 INTERNATIONAL STAFF

MCH-FP PHYSICIAN	P5	1	1	7060	7060
				SUBTOTAL	7060

3700 SUPPLIES AND DRUGS

CODE	ITEM	AMOUNT
3701	DRUGS	500
3703	HOSPITAL LAB	900
~~3708~~	~~LAB. SUPPLIES~~	~~0~~
3710	CLEANING SUPPLIES	100
3714	OFFICE SUPPLIES	300
3715	FOOD AND REFRESHMENT CLIENTS	3000
	SUBTOTAL	4400

3800 OTHER COSTS

PRINTING AND PUBLICATIONS		1500
LODGING		500
	SUBTOTAL	2000

*4800 INTERDEPARTMENTAL SERVICES

4802	TRANSPORT* DHAKA-~~MATLAB~~	200
4803	TRANSPORT MATLAB	500
4804	WATER TRANSPORT MATLAB	5000
4809	COMMUNICATION	1300
	SUBTOTAL	7000

0300	CAPITAL EQUIPMENT	
	COMPUTER EQUIPMENT	4000
	OFFICE FURNITURE	400
	SUBTOTAL	4400
	TOTAL DIRECT COST	80160
	MANAGEMENT, ADMINISTRATION, PERSONNEL & OTHER INDIRECT COST	24050
	* 31%	
	TOTAL PROJECT COST-MATLAB	105010

Ok
Shamima Ho
27/12/93

Justification:

This subcontract budget between ICDDR,B and JHU was drawn up to support the local field activities involved in conducting this study. A number of items neccessary for conducting this study are provided for in the cooperative agreement which JHU has with USAID for the support of this study, rather than in this subcontract. These items include the cost of study capsules, laboratory equipment and supplies.

REVIEWERS COMMENT

Most of the experiences so far with large dose Vitamin A prophylaxis programme have involved periodic distribution of high dose supplements as directed towards prevention of clinical eye signs. This is a new attempt to find out if supplement of ß-carotene capsule as a proxy for dietary Vitamin A supplement can also improve the Vitamin A status of the mothers and their breast fed infants. I believe preventing programmes with the objective of changing diets including (I) availability of Vitamin A rich foods through horticultur interventions (II) intervention programmes through control of infection and malnutrition and (III) improvement of income generating activities to enable the mothers to increase their purchasing power would possibly be the future strategies for decreasing childhood blindness and other morbidity in most of the third world countries.

The study proposed, is a very important one, though I think it will give us a limited answer in terms of the long term benifits to the children beyond the age of 9 months when they will not be protected anymore by mother's milk. Specially, as it is well known that a significantly large number of children between ages 1-5 years suffer from eye diseases due to Vitamin A deficiency, diarrhoea and respiratory tract infection. Therefore, attempt should be taken also to find out if this has got any long term effect in terms of reducing morbidity and mortality after this 9-months period. Otherwise, again it will be only an interim measure and no long term solution will be achieved.

Since the conversion of ß-carotene to vitamin A is not clearly known, I think it would not be a waste to do a small pilot study on a group of mothers to find out if serum and breast milk vitamin A levels are comparable between the two groups receiving ß-carotene and high potency capsule.

Finally I would recommend this application because, the information generated from this research would be helpful for formulation of a national programme for improvement of nutritional status of the children of Bangladesh.

cc: - Ms Amy Rice

Review 2

In general, the study is carefully designed, well-controlled and if conducted according to plan will serve the objectives and show scientific merit. The study will provide important data to be used for planning effective vitamin A intervention programs. There are a few minor points of comments and suggestions for investigators' consideration:

1. The proposal did not specify the reason why enrolled mothers are not matched to assure comparability of key baseline characteristics (age, parity, nutritional status, socioeconomic status etc) prior to assignment to treatment groups. My understanding is that the population are quite uniform or will be screened to be so.

2. The investigators admit that dietary vitamin A intake is likely to a be a confounder for the results (page 23) and seasonal variation is known. If so, why not consider collect dietary data at least once per season and if feasible, try 24- hour recall plus weighing method in a subsample population. Although this is not the main focus of the study, it may help to explain up to a certain extent the outcome especially the so-called "placebo effect".

3. If infant's blood collection is done by fingerprick, caution must be taken for interstitial fluid contamination.

cc: Amy Rice

Response to Reviewers' Comments

Review 1

Comment: The reviewer suggests that the cohort of children be followed for longer than the nine month study period to examine any effects supplementation has on longterm morbidity and/or mortality.

Response: The sample size for this study (216 infants-divided between three groups) was chosen to detect differences in biochemical measures between groups, not differences in morbidity or mortality. While information on morbidity and mortality among this cohort could be abstracted from MCH-FP records past the nine month follow-up period of this study, the resulting data would have very limited power to examine any differences. For this study morbidity and mortality data will only be collected for descriptive purposes during the nine month follow-up period.

Comment: The reviewer suggests that a pilot study be done to determine whether serum and breastmilk vitamin A levels are comparable between the two groups receiving ß-carotene and the high potency retinol capsule.

Response: This is planned as part of the baseline assessment. Breastmilk samples will be collected from all women at baseline and analysed for their retinol and ß-carotene content. Blood samples will be collected from half of the women (36) in each group at baseline and analyzed for retinol and ß-carotene content. This data will be sufficient to establish group comparability at baseline.

Review 2

The responses are numbered according to the reviewer's comments.

1. An explanation has been incorporated into the protocol. The population is reasonably homogenous and considering that the randomization sheme is already complex, any marginal benefits that would be obtained by matching the study participants on baseline characteristics would be outweighed by the logistical difficulties involved in attmepting to do so.

2. A 24 hour recall interview will be conducted with each woman four times throughout the study, at baseline, three, six and nine months. This will be sufficient to establish group comparability during the study period and describe the dietary patterns in the population.

3. Infant's blood collection will be done by heelprick and care will be taken to avoid interstitial fluid contamination.

Informed Consent Statment
RETIBETA Study

This is a special health project being carried out by MCH-FP and Johns Hopkins University in the United States. This project has been approved by the Cholera Hospital (ICDDR,B).

The purpose of this project is to see whether giving you special vitamin capsules will help you and your baby. We know that some of the things you eat go into your breastmilk. If we give you vitamins, they may go into your breastmilk and your baby will drink them. The Cholera Hospital (ICDDR,B) will use the results of this study to help decide if these kinds of vitamins should be given to women in Matlab and other parts of Bangladesh to help them and their babies. You are being asked to participate in this study because you live in the MCH-FP intervention area and have recently had a baby.

If you decide to participate, the MCH-FP community health workers will provide you with vitamin capsules for you to take every day for the next nine months. We will also ask you and your baby to come to the Matlab Cholera Hospital for a special health exam visit four times in the next year.

During your visits, the following things will happen:

1. The doctor will exam and treat you for any problems you may have.

2. Your height and weight and arm circumference will be measured.

3. The doctor will examine your eyes to determine how healthy they are. For this exam the doctor will momentarily touch a special piece of paper to the surface of your eye. By looking at this paper under a microscope we can learn about the health of your eyes.

4. A female clinic worker will collect a sample of your breastmilk. We will send it to the laboratory in Dhaka to see how nutritious it is.

5. A clinic worker will ask you questions about the foods you eat.

6. During two of your four visits, the doctor will give you another vitamin capsule and after several hours he will collect a small blood sample to see if the vitamins made your blood more healthy. The blood will be collected from your arm. The total volume of blood collected at one time will be about 3 cc.

The steps in your baby's exam will be:

1. Your baby will be examined and treated for any problems it may have.

2. The length and weight of your baby will be measured.

2. A clinic worker will ask about the foods your baby eats.

4. When your baby is six months old, a small blood sample will be taken from the heel. The blood will be examined at a laboratory in Dhaka to see how healthy it is.

For each of the four visits, we will provide transportation for you and your baby to come to the Matlab clinic. You will also receive tea and lunch during your visits.

Your participation in this study is voluntary and you may withdraw at any time. If you choose not to participate, you will continue to receive MCH-FP services as you always have. If you have any questions about this project, you can ask your local community health worker or contact Dr. Andres de Francisco or Amy Rice at the Matlab clinic.

After hearing this explanation of the RETIBETA project, I agree to voluntarily participate. I understand that I may withdraw at any time without any penalty.

_____ _____, 1994

Mother's left thumbprint Day/Month

_____ _____

Mother's name Witness' name

BASELINE QUESTIONNAIRE
(for the mother)

RETIBETA ID: _____ Date: _____

Mother's name: _____ Father's name: _____

MCID: _____ Village: _____

MRID: _____ Bari: _____

Baby's name: _____

Baby's date of birth: _____

Baby's sex: M F

Pregnancy events:

Any special difficulties?

nightblindness in preg?

nightlblindness in previous pregnancies?

Events surrounding birth:

Where?

Who attended?

Any special difficulties?

Infant feeding practices:

When did you start breastfeeding?
same day
next day...

Did you feed the child colostrum?

What other liquids or foods have you given the child since birth?

24 Hour Recall Interview
(with mother)

RETIBETA ID _____ Date _____

1. Did any special event happen yesterday?
   Yes [ ] 1
   No [ ] 2 --> Go to 4.0

2. Which event or events?
   Guests [ ] 1
   Fasting [ ] 2
   Marriage [ ] 3
   Other [ ] 4
   _____

3. Did this alter your diet?
   Yes [ ] 1
   No [ ] 2

4. Other than the RETIBETA study capsules, did you take any type of vitamin pill or capsule?
   Yes [ ] 1
   No [ ] 2 --> Go to Food List

5. What type of capsule?
   _____

Food List

Time/ Activity	Food item	Method of Preparation	Described Amount		Amount (g)		Food Code

RETIBETA ID Number [__ __ __]

Time/ Activity	Food item	Method of Preparation	Described Amount		Amount (g)		Food Code

Interviewer ______________	[__ __]	Date: __ / __ / __
Reviewer ______________	[__ __]	Date: __ / __ / __
Data Entry ______________	[__ __]	Date: __ / __ / __

RETIBETA
FORM SI0

INFANT SUPPLEMENTAL FEEDING QUESTIONAIRRE

Mother's name: _____ RETIBETA ID: _____

Baby's name: _____ Date: _____

Baby's birthdate: _____

1.0 Has your baby been sick during the past week?

[ ] yes
[ ] no --> go to 3.0

2.0 Has your baby eaten normally during the past week?

[ ] yes
[ ] no

3.0 Has your baby eaten or drank anything other than breastmilk during the past week?

[ ] yes
[ ] no --> STOP

Please check all the foods that your baby has eaten during the past week.

Liquids:

ITEM	none	1-3 wk	4-6 wk	1 day	2 day	3 day	4+ day	vol tsp.
water								
sugar water								
rice water								
tea								
other								

Porridges:

ITEM	none	1-3 wk	4-6 wk	1 day	2 day	3 day	4+ day	vol tsp.
other								

Fruits:

ITEM	none	1-3 wk	4-6 wk	1 day	2 day	3 day	4+ day	vol tsp.
other								

Vegetables:

ITEM	none	1-3 wk	4-6 wk	1 day	2 day	3 day	4+ day	vol tsp.
other								

etc...

Vitamin capsules/medicines

[ ] yes _____
[ ] no

RETIBETA
FORM MK0

BREASTMILK SAMPLE QUESTIONNAIRE

Mother's name: _____ RETIBETA ID: _____

Baby's name: _____ Date: _____

Baby's birthdate: _____

1.0 Time at start of interview [___:___]

2.0 How long has it been since you fed your baby from your left breast?

[ ] > 2 hours
Collect the sample from the left breast. --> go to 4.0
[ ] < 2 hours
[ ] don't know
[ ] left breast rarely used to breastfeed

3.0 How long has it been since you fed your baby from your right breast?

[ ] > 2 hours
Collect the sample from the right breast. --> go to 4.0

[ ] 1.5 - 2.0 hours
Add 1/2 hour to the start of interview time and record [___:___]
Wait until then.
Collect the sample from the right breast. --> go to 4.0

[ ] 1.0 - 1.5 hours
Add 1 hour to the start of interview time and record [___:___]
Wait until then.
Collect the sample from the right breast. --> go to 4.0

[ ] 0.5 - 1.0 hour
Add 1 1/2 hours to the start of interview time and record [___:___]
Wait until then.
Collect the sample from the right breast. --> go to 4.0

[ ] < 0.5 hours
Add 2 hours to the start of interview time and record [___:___]
Wait until then.
Collect the sample from the right breast. --> go to 4.0

[ ] don't know
Add 2 hours to the start of interview time and record [___:___]
Wait until then.
Collect the sample from the right breast. --> go to 4.0

4.0 Time sample collection began [___:___]

5.0 Sample taken from
right breast [ ]
left breast [ ]

6.0 Volume of sample
_____ mls

ANTHROPOMETRIC MEASUREMENTS

Mother's name: _____ RETIBETA ID: _____

Baby's name: _____ Date: _____

Baby's birthdate: _____

1.0 Baby's weight [___,__] kg

2.0 Baby's length [___,__] cm

3.0 Mother's weight [___,__] kg

4.0 Mother's height [___,__] cm

5.0 Mother's mid-upper arm circumference [___,__] cm