

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator DR. G FUEHS

Trainee Investigator (if any) _____

Application No. ~~95-000~~ 95-011

Supporting Agency (if Non-ICDDR,B) OMNI

Title of Study: Dietary Fat and Infection Project status:

- Relationship to Vitamin A status of Women () New Study
- and their infants, Breastmilk Retinol/ () Continuation with change
- Carotenoids, and Dietary Intake Methodology. () No change (do not fill out rest of form)

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

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

2. 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

3. 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

4. Are subjects clearly informed about:
- (a) Nature and purposes of study Yes No
 - (b) Procedures to be followed including alternatives used Yes No NA
 - (c) Physical risks Yes No
 - (d) Sensitive questions Yes No
 - (e) Benefits to be derived Yes No
 - (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 NA

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).
 - Protocol (Required)
 - Abstract Summary (Required)
 - 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)
 - Informed consent form for subjects
 - Informed consent form for parent or guardian
 - Procedure for maintaining confidentiality
 - 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.

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

Dr. G. Fuhs
Principal Investigator

Trainee

DIETARY FAT AND INFECTION: RELATIONSHIP TO VITAMIN A STATUS OF WOMEN AND THEIR INFANTS, BREASTMILK RETINOL/CAROTENOIDS, AND DIETARY INTAKE METHODOLOGY

1. HYPOTHESES

1. Reduced absorption of dietary vitamin A and carotenoids due to low dietary fat intake is a cause of a poor vitamin A status despite the apparent adequate intake of vitamin A-containing foods.
2. Decreased bioavailability of vitamin A due to inadequate dietary fat has an adverse effect on maternal vitamin A status and breastmilk retinol concentration, and therefore potentially the status of their infants.
3. Diarrheal disease and respiratory tract infections (RTI) result in poor vitamin A status despite the apparent adequate intake of vitamin A-containing foods.
4. The frequency and/or duration of diarrheal disease and RTI is associated with maternal vitamin A status and breastmilk retinol concentration, and therefore the status of their infants.
5. IVACG's Simplified Dietary Assessment (SDA) to assess risk of vitamin A deficiency (VAD) in children can be modified and used to assess risk of VAD in pregnant/lactating women.
6. Accuracy of the SDA to assess vitamin A status is effected by dietary fat.
7. Accuracy of the SDA to assess vitamin A sufficiency is effected by the frequency/duration of diarrheal disease and RTI.
8. The accuracy of the SDA to assess risk for VAD is improved by adjustment of scores for dietary fat and/or diarrheal disease/RTI.

2. OBJECTIVES

1. Determine if an intervention to increase dietary fat will result in improved maternal vitamin A status, breast milk retinol/carotenoid concentration, and vitamin A status of their breastfeeding infants.
2. Determine the relationship between diarrheal disease/respiratory tract infections and maternal vitamin A status, breast milk retinol/carotenoid concentration and the vitamin A status of their breastfeeding infants.
3. Determine if the IVACG SDA can be modified to assess risk of VAD in populations of pregnant/lactating women.
4. Determine the influence of dietary fat and diarrheal disease/RTI on results of the SDA to assess vitamin A status.
5. Determine if adjustment for dietary fat and/or diarrheal disease/RIT will result in improved accuracy of vitamin A dietary methodologies.

3. BACKGROUND INFORMATION

The International Vitamin A Consultative Group (IVACG) has proposed a simplified dietary assessment (SDA) as a dietary assessment method to identify and monitor groups at risk for inadequate vitamin A intake, and therefore at risk for VAD.¹ The SDA assesses the intake of a single nutrient, vitamin A, thereby avoiding many of the problems of whole diet surveys.² In Northern Thailand we found that IVACG's SDA accurately assigned risk for VAD as compared to blood retinol criteria.³ Further, conjunctival impression cytology (CIC) was in agreement with the SDA using CIC prevalence criteria in the communities in which both tests were performed.^{4,5} We concluded that the SDA had significant potential as a practical, inexpensive, non-invasive field tool successfully implemented by non-specialists to assess risk of populations for inadequate vitamin A intake and consequent VAD. The SDA is designed to particularly target preschool age children in the assessment of risk for VAD, and to date the reported experience with the SDA reflects this. However, other groups are also at risk for VAD, an important vulnerable group being pregnant and lactating women. VAD in this group has repercussions for not only for the women themselves but potentially for their newborn and

breastfeeding infants as well. *Successful application of the SDA in pregnant/lactating women would result in an important noninvasive rapid field tool to determine risk of VAD in this susceptible group and potentially their infants.*

While the SDA has shown significant promise, validation trials are preliminary and results have been inconsistent.⁶ Indeed, our own experience subsequent to our initial report suggests that other confounding variables effect the accuracy of the SDA.⁷ While this observation could be the result of an overestimation of provitamin A intake by the SDA, an alternative or additional explanation is that other variables which effect vitamin A status and which are independent of vitamin A intake might be important determinants. Regarding the latter, it is probably relevant that investigators recently reported no improvement in vitamin A status in lactating women despite the well-controlled administration of a metered dose of provitamin A via a vegetable wafer.⁸ Within this context certain factors might be predicted to exert a pivotal effect on the accuracy of all such methods. *In particular, poor absorption of ingested dietary carotenoids and vitamin A would be expected to result in underestimation of risk of VAD as measured by dietary methodologies.* Improved accuracy and further validation trials of these new methodologies is essential if this or other similar methodologies are to achieve widespread use.

Dietary fat is essential for maximal utilization and absorption of ingested vitamin A and carotenoids.⁹ A low intake of fat is typical in the same populations with VAD around the world.^{9,10} That low dietary fat might contribute to the genesis of VAD by limiting the bioavailability of vitamin A and carotenoids is therefore a meaningful possibility. Although fat supplements have been shown to improve carotenoid absorption in studies involving small numbers of children studied under metabolic ward conditions, population-based studies are lacking in general and to our knowledge are nonexistent in the target group we propose to study.^{9,10,11} *If increasing fat intake is established to independently effect population vitamin A status, this would have relevant programmatic implications in the prevention and amelioration of VAD.*

Women in developing countries have an average daily intake of vitamin A that is less than half and average serum retinol that is 70% of intake and serum retinol, respectively, of women in developed countries.¹² The vitamin A status of most newborns is marginal, and those whose mothers have inadequate vitamin A intake appear to be at particular risk.^{13,14} In a recent study of 85 Bangladeshi primarily breastfed infants with a mean age of 6 months, we observed 60% and 65% to have a low vitamin A status according to serum retinol and RDR, respectively.¹⁵ In another recently completed study we reported that supplementation of lactating women with a single 300,000 IU dose of vitamin A significantly improves the vitamin A status of mothers and their breastfed infants, and results in a decrease in infant mortality.^{16,17} It might be relevant that fat intake by lactating women in developing countries, as with their children, is reported to be very low.^{18,19,20} Relatively few studies have compared breastmilk vitamin A concentration with intake of vitamin A by lactating women.¹² *If increasing fat intake in women with low dietary fat improves vitamin A bioavailability, it would be predicted that an improvement in maternal and infant vitamin A status as well as breast milk retinol and carotenoid concentrations would result.*

Coexistent infection is also a potential determinant of vitamin A status in children. Reductions in serum retinol have been observed in connection with childhood infections due to increased requirements and/or urinary loss.^{21,22,23,24,25} Intestinal parasitosis is capable of impairing absorption of vitamin A and carotenoids.^{26,27,28} These

conditions of morbidity that result in abnormal needs, abnormal loss, or reduced bioavailability of vitamin A are independent of vitamin A intake and are not considered in the scoring systems of dietary vitamin A assessment methodologies. *It is conceivable therefore, that this could result in an underestimation of risk of VAD as determined by dietary methodologies.*

We expect the results of the proposed study to 1) ascertain the importance of dietary fat and infection on the vitamin A status of pregnant/lactating women and subsequently their breastfeeding infants. Further, if the hypotheses are correct, we expect the results will 2) determine the validity of dietary methodology to assess risk of VAD in pregnant/lactating women and, because breastmilk concentration is lower in women with deficiency, potentially their breastfeeding infants. 3) enable the development of precise, simple methods to incorporate fat intake and/or infection factors into the SDA methodology to reflect issues of vitamin A bioavailability and metabolism and to therefore improve its accuracy and efficacy, and 4) provide a rationale for further study of increasing dietary fat as an additional strategy to improve vitamin A status in certain populations.

4. METHODS

4.1 Study Population: \approx 600 pregnant/lactating women and their infants will be recruited from \approx 30 villages of Matlab thana of Chandpur District, the principal field station for the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). Women will be enrolled at five to 7 months gestation, and completion of the study for each mother/infant pair will be when the infant reaches 6 months of age. As is typical in rural Bangladesh, the villages proposed to participate in the study are known to have a vitamin A problem as well as a diet which is very low in fat. As a result, the vitamin A status of the women, breastmilk retinol concentration, and the vitamin A intake by breastfeeding infants is also low.²⁹ These villages have also been identified to have a greater intake of provitamin A-containing foods compared to typical villages as the result of the "spillover" of a campaign to increase the intake of green leafy vegetables in villages from the surrounding areas. Further, unlike the children of these villages, the adults do not participate in a program of periodic dosing with vitamin A capsules. We accordingly believe this is a suitable population for testing of our hypotheses and our proposed intervention.

4.2 Dietary Assessment:

- *The IVACG simplified dietary assessment*

The simplified dietary assessment that we have previously evaluated will be administered to the women and scored according to the IVACG guidelines.^{1,3} With the SDA, nutrition interviews are conducted after food sources of vitamin A (markets, household gardens, etc) in the community are identified and dietary patterns and preparation methods are determined. Portion sizes (small, medium, or large) of food are estimated and the vitamin A content for typical portion sizes are calculated. A "Vitamin A Score" is then assigned to each food. For this purpose we intend to use the food composition table for use in South Asia developed by the National Institute of Nutrition of Hyderabad, supplemented with data from other culturally appropriate food composition tables and the Helen Keller International database for Bangladesh in particular.³⁰ A pre-coded questionnaire is constructed based on the foods that together contributed 90 percent or more to the vitamin A intake as well as their frequency of consumption.

Intake information is obtained by both 24-hour dietary recall and by history of the usual pattern of consumption of vitamin A-rich foods during the previous one month to account for daily variations in vitamin A intake. The data obtained in this fashion is used

to calculate a consumption index (CI) and usual pattern of food consumption (UPF). The CI and UPF will then categorize the communities of women into risk level based on the FAO/WHO recommended dietary intake and assigned a risk score of high, medium, or low risk of dietary vitamin A inadequacy.

- *Dietary Fat intake will be quantitated by monthly 72 hour whole diet recall.*

Fat intake will be quantitated by whole diet recall methodology which, in addition to fat, will also provide quantitative data for protein and energy.

4.3 Biochemical Assessment: 250 μ L of plasma will be obtained by "fingerstick" (women) or "heelstick" (infants) for determination of retinol concentration and the concentrations of the main pro-vitamin A carotenoids in blood (β -carotene, β -cryptoxanthin, α -carotene) by HPLC.^{31,32} The carotenoids are assessed because the vitamin A in the staple diet of our population, like most populations with VAD, is derived from provitamin A plant sources.

Random five ml breastmilk samples will be collected monthly from mothers 1-8 months postpartum. Sample collection methodology and retinol concentration interpretation will be according to the 1994 WHO instructions.³³ Although breastmilk retinol and fat concentration is variable and dependent on many factors, a random sample is best for the purpose of assessing vitamin A status in a population (in contrast to an individual) assuming that samples are collected throughout the day and at varied periods following the last feed.³³ Simultaneous analysis of breastmilk retinol and carotenoid concentrations will be performed after saponification by HPLC according to a modification of the method of Giuliano et al. which has previously been established in our laboratory.³⁴

4.4 Anthropometry: Anthropometric measurements (mid upper arm circumference [MUAC]) of pregnant women, (weight, weight for height, body mass index [weight/height²]) lactating women, and (weight, weight for length, midarm circumference) their infants will be recorded indicators of general nutritional status. Measurements will be compared to age- and sex-appropriate international standards.

4.5 Assessment of Infection Morbidity: Health workers will visit the households every other week and record episodes of fever, diarrheal disease and respiratory tract disease symptoms reported since the previous visit. Diarrhea is defined as stools that are unusually loose or frequent for that study subject or any stools that contain blood; passage of a minimum of three loose or watery stools or any number of stools containing blood in a 24-hour period will be required.³⁵ New episodes are defined as three intervening diarrhea-free days and chronic diarrhea defined as diarrhea that persists > 2 weeks.³⁵ Episodes of sore throat, hoarseness, cough, or difficulty breathing will also be recorded. An ARI is considered a new episode if the subject is symptom-free for a minimum of 2 weeks preceding an illness.³⁶ Need for a clinic visit or hospitalization, duration of hospitalization, and duration of illness is recorded as additional measures of severity of diarrheal or respiratory illness.

4.6 Stool Microscopy For Parasites: A stool sample will be obtained from a 50% random subsample of women after the intervention period and be examined by microscopy after formalin ether concentration.³⁷ The stool concentration technique results in a better yield and is therefore more sensitive than direct exam. Individuals identified with pathogens will be treated. Baseline testing will not be done since results would not be available until later in the study period, and treatment during the dietary intervention period would introduce a confounding variable.

4.7 Dietary Intervention: One-half of women at five to seven months gestation will be randomized by village and provided with soybean oil (test group) for a period of 8-10 months depending on stage of gestation at enrollment. Pregnant/lactating women in this group will be instructed to mix 10 ml of soybean oil (9 g fat) in their rice (eaten at each meal) at each meal (2 meals/day). The study is designed to supplement the diet in this fashion rather than instructing women to add oil during household food preparation to ensure a measured "dose" of oil in order to optimize our ability to test the study hypothesis. All members of the household will receive the supplement to maximize compliance to the protocol. Preparation of the study subjects will be achieved through focus group and individual education will be provided to ensure understanding of the protocol and to maximize compliance. Health workers will make every other weekly home visits during the intervention to reinforce the protocol. The supplement quantity is extrapolated from previous metabolic ward studies.^{9,11}

5. SCHEDULE OF EVALUATION AND TESTING (See Appendix)

Staff and study site preparation is scheduled to occur during the first two months. Enrollment will occur over the ensuing ten months since conservative estimates indicate an average expected subject enrollment of 60 subjects per month. Data entering and specimen testing will be performed over the course of the study beginning with the data collection period. Fat intake (in addition to protein, energy intake) will be assessed via whole diet recall eight times for each subject over the course of the study. Vitamin A status will be assessed by the SDA upon enrollment, at birth of the infant, and at 1, 3, and 6 months postpartum. Maternal plasma retinol will be determined coincident with the SDA except at birth since it is not feasible for the field team to be present at birth and because of concern that collection of the blood specimen at that time would be considered by the subject as too invasive. The dietary intervention in the study group will begin upon enrollment and end at study completion (6 months postpartum).

6. STATISTICAL ANALYSIS

Previous surveys indicate a prevalence of low retinol ($<0.07 \mu\text{mol/L}$) in our sample of 25% to 50%. To detect a 50% decrease in the prevalence of low retinol at a 5% significance level with a power of 90% due to our intervention, we estimate the need for 200 women in each of the two groups based on the 25% baseline prevalence.³⁸ We propose an enrollment goal of ≈ 300 women per group since we expect a "dropout" rate of $\approx 25\%$ based on prior experience with similar populations. Relationships between variables (eg, frequencies) will be examined by chi-square test and group comparisons of continuous data (eg, mean values) by Student's *t* test. Principal outcome variables will be maternal plasma retinol/carotenoids, breastmilk retinol/carotenoids, and infant plasma retinol/carotenoids. The sensitivity, specificity, and predictive value of selected variables will be assessed with the plasma retinol or breastmilk retinol as the "reference standard" depending on the interaction being investigated. For plasma criteria, vitamin A status will be characterized as deficient, low, or adequate ($<0.35 \mu\text{mol/L}$, $<0.70 \mu\text{mol/L}$, and $\geq 0.70 \mu\text{mol/L}$ respectively).^{33,39} Regarding breastmilk concentration, a public health problem is defined according to the prevalence of retinol concentrations $\leq 1.05 \mu\text{mol/L}$ (mild, $<10\%$; moderate, $\geq 10\% - <25\%$; and severe, $\geq 25\%$).³³

Regression analysis will be used to assess interactions between dependent variables and multiple independent variables, the strength of which will be determined by standard correlation tests. Groups will also be stratified for analysis as necessary (frequency or type of infection, etc).

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APPENDIX. SCHEDULE OF EVALUATION AND TESTING

STUDY TIMELINE

Activity	Month			
	1 - 2	3 - 12	13 - 20	21 - 24
Staff and site preparation	X			
Subject enrollment		X		
Data collection		X	X	
Dietary Intervention		X	X	
Data entering and analysis, specimen testing		X	X	X

DATA COLLECTION

Activity	Enrollment	Birth	Postnatal Month						
			1	2	3	4	5	6	
Pregnant/lactating Woman									
-Dietary assessment SDA	X	X	X		X				X
-Whole diet recall	X	X	X	X	X	X	X	X	X
-Anthropometry	X		X		X				X
-Plasma retinol/carotenoids	X		X		X				X
-Breastmilk retinol/carotenoids			X		X				X
-Stool ova + parasite exam									X
-Dietary Intervention	X	X	X	X	X	X	X	X	X
-Morbidity assessment (every two weeks)	X	X	X	X	X	X	X	X	X
Infant									
-Plasma retinol/carotenoids									X
-Anthropometry			X						X

SDA, simplified dietary assessment for vitamin A intake.

COLLABORATORS OF PROPOSED PROJECT:

K.M.A. Aziz: Earned a Master's degree (1960-61) in Sociology from the University of Dhaka, and M. Phil. (1974-76) and Ph.D (1977-81) in Rural Sociology; and Sociology and Anthropology respectively from the university of Rajshahi. He joined ICDDR,B as Sociologist in 1961 and has worked in many key research and administrative positions. He is currently a Senior Scientist, Community Health Division. Dr. Aziz has published over 30 scientific articles author or co-author in international journals. Has participated and presented scientific papers in numerous international scientific conferences held in South Asia, U.S.A., Canada, and Africa. At present he is the Principal Investigator of an on-going protocol of ICDDR,B entitled "Development and implementation of nutrition education strategy for promotion of Beta-carotene rich foods as a source of vitamin A in children".

Mohammad Yunus, MD, MSc. Mohammad Yunus is a Scientist and Coordinator of Matlab Health and Research Centre of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). He obtained his medical graduation from Dhaka University, Bangladesh in 1968 and joined ICDDR,B the same year. He obtained his Master of Science degree in Community Health in Developing countries from London School of Hygiene and Tropical Medicine in the year 1982. He has been associated with many studies in diarrhoeal diseases and nutrition in Matlab and conducted the oral therapy field trial as principal investigator. He is currently a collaborative investigator of an ongoing protocol of ICDDR,B entitled "Development and implementation of nutrition education strategy for promotion of Beta-carotene rich foods as a source of vitamin A in children".

M. A. Wahed, B.Sc.: Head of the Biochemistry and Nutrition Branch of the Centre. He earned his B.Sc (major in Chemistry) from Rajshahi University in 1966. Subsequently he did postgraduate course in Nutrition Biochemistry from United Nation University. He completed various training programmes (1) Clinical Biochemistry from University of Western Australia in 1985 (2) Nutrition Biochemistry Techniques at MRR, Dunn Nutrition Lab in 1988 (3) Quality Control Management at BMDC, 1989 and (4) Vitamin A methodology workshop at IOWA State University in 1992. He has to his credit both as Principal and Co-author about 50 publications. The results of his work have been presented in over 25 meetings both at home and abroad. He is currently a Principal Investigator on Vitamin A programme supported by USAID. For the proposed study he and his staff will be involved in laboratory activities.

Andres de Francisco, MD, PhD: Dr. De Francisco is currently the Project Director of the Matlab Maternal and Child Health and Family Planning (MCH-FP) Programme at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) where he has been engaged as a principle investigator in vitamin A research, among other activities. Previously, he was Senior Scientist, British Medical Research Council (MRC) Laboratories, The Gambia, West Africa for three years. He earned his medical degree at Rosario University, Bogotá, Colombia in 1983, and a Masters in Clinical Tropical Medicine from the London School of Hygiene and Tropical Medicine, 1986. Subsequently he earned a Diploma in Tropical Medicine and Hygiene (Royal College of Physicians, London; 1986), and a Masters in Community Health in Developing Countries (London School of Hygiene and Tropical Medicine; 1987), and his PhD in Medicine (University of London; 1994.)

Revised Dec 20, 1994

OMNI PROPOSAL: VITAMIN A AND DIETARY FAT		
1. Personnel		
George Fuchs, MD (P.I.)	20% effort	8,355
K.M.A. Aziz, Ph.D (Co-P.I.)	25% effort	11,481
M. Yunus, MBBS, MSc, (Co-Investigator)	10% effort	4,109
M.A. Wahed, BSc (Co-Investigator)	10% effort	3,100
Andra de Francisco, MD, Ph.D. (Consultant)		Nil
	Subtotal	27,045
Field Team		
Nutritionist (1)		8,520
Supervisor (1) 60% effort		9,605
Health Assistant (3)		26,856
Community Health Workers (5)		7,200
Boatman (6)		8,640
Porter/Messengers (2)		2,520
Trainee Physician (1)		4,800
Trainee Sociologist (2)		4,000
	Subtotal	72,141
Data Management		
Programmer (1)		1,836
Data Processing Assistant (1)		7,200
Data Entry Technician (1)		5,448
Coder (1)		5,448
	Subtotal	19,932
Miscellaneous personnel		
Office Clerk (1)		4,000
Clinic Staff		1,000
Research Asst (1) for retinol assays		3,000
	Subtotal	8,000
2. Laboratory		
Plasma retinol/carotenoids	\$5.20 per test x 5 test per mother-infant pair x 600	15,600
Breastmilk retinol/carotenoids	\$5.20 per test x 3 test/mother x 600	9,360
Stool microscopy	\$2 per test x 600	1,200
Misc. laboratories supplies		1,500
	Subtotal	27,660
3. Supplies		
Soybean Oil		7,000
Computer (for Data Management)		4,500
Fuel and Transportation for Field team		15,500
	Subtotal	27,000
4. Subtotal Direct Cost		181,778
5. Indirect Cost (10%)		18,178
6. TOTAL COSTS		199,956

B.H.
8/5/95

Title: Dietary fat and infection; relationship to vitamin A status of women and their infants breastmilk retinol/cartinoids, and dietary assessment methodology.

Summary of Referee's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

Rank Score

	High	Medium	Low
Quality of Project		✓	
Adequacy of Project Design			✓
Suitability of Methodology	✓		
Feasibility within time period	✓		
Appropriateness of budget	✓	-	-
Potential value of field of knowledge	✓		

CONCLUSIONS

I support the application:

- a) without qualification 1 1
- b) with qualification
 - on technical grounds 1 1
 - on level of financial support 1 1

I do not support the application 1 1

Name of Referee: Dr. Faruk Ahmed.

Signature: *F. Ahmed*

Associate Professor

Date: ..24-4-95..

Position:

Institution: Institute of Nutrition and Food Science.
University of Dhaka

Detailed Comments

Please briefly provide your opinions of this proposal, giving special attention to the originality and feasibility of the project, its potential for providing new knowledge and the justification of financial support sought; include suggestions for modifications (scientific or financial) where you feel they are justified.

(Use additional pages if necessary)

Title: Dietary Fat and Infection : Relationship to vitamin A status
of women and their infants, Breastmilk retinol/carotenoids, and
and dietary intake methodology.
PI: Dr. George Fuchs
Reviewer: Dr. Faruk Ahmed.

The research proposal seeks to investigate a number of important issues which has significant public health implications.

The study findings will strengthen the existing knowledge of the importance of dietary fat, and infection in relation to vitamin A status, while the findings in relation to validation of IVACG's Simplified Dietary Assessment technique to assess risk of vitamin A deficiency in pregnant/lactating women will add new knowledge in the field of vitamin A nutrition.

However, the study proposal has certain design flaws which need to be considered before implementation of the project:

(a) It has been emphasized that a number of factors may influence bioavailability of provitamin A (carotenoid) in foods. Among which the nature of matrix in which carotene is embedded in a particular food is very important.

How the effect of this potential confounding factor will be dealt during the assessment of the effect of dietary fat intake (absorption modifier) in improving vitamin A status has not been mentioned.

Cont'd -2

(b) One of the major concern about the study design is the method of intervention. The subjects of the experimental group will be instructed to mix 10 ml raw soybean oil with the rice of each of their two daily meals for a period of 8-10 months. There is considerable doubt as to the acceptance by the subject of such a dietary modification to their normal practice. Besides, the duration seems too long for this type of supplementation study in free living subjects.

The mode of supplementation is therefore likely to reduce compliance and reliability of data and consequently may lead to erroneous conclusion. The investigators should consider a more culturally acceptable method of oil supplementation.

(c) Pregnancy is a state of physiological stress, and there are reports that serum retinol level declines with the progress of pregnancy in poor population. Further, no normative values of serum retinol are available for pregnant women with which one can evaluate the degree of vitamin A deficiency in order to validate the IVACG's SDA method to assess the risk of vitamin A deficiency in this population. Pregnant women as a study population seems an inappropriate group to perform the validation study of IVACG's SDA to assess the risk of vitamin A deficiency. The study should concentrate on lactating women rather than both pregnant and lactating women.

(d) Vitamin A available from food is affected by the method of preparation. Consideration of food processing and cooking loss is essential for interpretation of dietary intake information in terms of the bioavailability of dietary vitamin A and its likely impact on vitamin A status.

The food composition tables (both NIN or HKI database) which is referred to be used to calculate vitamin A intake are based on edible portion of raw foods and thus may result an overestimation of provitamin A intake by the IVACG's Simplified Dietary Assessment method. How will this issue be resolved while doing the validation study of IVACG's SDA method.

(e) Intestinal parasites may cause impaired absorption of vitamin A and carotenoids. However, the study design does not include any baseline stool examination or treatment for intestinal parasites. It is not clear how examination of stool of a subsample for parasites after completion of intervention will help to interpret the findings.

F. Ahmed
24.4.95

REVIEWERS COMMENTS

Global Impact

- Relevant
- Good site

Knowledge of Proposed Work and Past experience of Principal Investigators

- Experienced, lab available
- Excellent

Methodological Approach

- Approach seems sound and achievable
- Good Idea if focus only on the effect of fat on vitamin A on bioavailability and status
- Carotene added to soy oil maybe a more effective way.

Research environment

- Resources and expertise available.

OMNI PROJECT:RESPONSE TO REVIEWER

Reviewer #1

- a) The reviewer has expressed concern about one particular variable (matrix) as a potential confounding factor that effects provitamin A bioavailability. Actually, several factors exist that have the potential to influence the bioavailability of provitamin A. We believe that it is not possible to control every potential variable. The proposed study aims to intensively study one of the more important potential influences, i.e. dietary fat. It is expected that the role of adequate dietary fat can be isolated from other confounding variables through the intervention in which dietary fat is manipulated in half of the study population.
- b) In response to the concern about the method of introducing oil into the diets, we are not in agreement with the reviewer. Based on fairly extensive experience of the Co-PI with this population, we have reason to believe the method is culturally appropriate, feasible, and will achieve the desired aim of increasing dietary fat. Even so, we intend to field-test this method prior to implementation. We have also considered alternative interventions to increase dietary fat if this proves necessary. Compliance to the study protocol will be reinforced and assessed during the scheduled contacts with the study participants.
- c) The reviewer points out the variability in vitamin A metabolism and vitamin A assessment by serum retinol in individual pregnant women. The reviewer is correct that serum retinol declines in many pregnant women, and that normal values are not clearly defined in this group of humans. To some extent this is also true for all people, including children, in which blood retinol does not necessarily correlate with body retinol. This is the reason for the interest in other more specific and sensitive tests such as the RDR and modified RDR. However, and of importance, what constitutes an *abnormal* retinol value is better understood (eg., $<.35 \mu\text{mol/L}$) during pregnancy as well as for other populations.

For the purposes of determining the effect of the intervention, group differences will be assessed by comparisons between the intervention and control groups of mean retinol as well as the difference in distribution of retinol values within each group. This is the identical approach to assess these issues in other groups (children, adults). In this way, the risk of vitamin A deficiency in a group of women can be categorized.

With regards to the evaluation of the IVACG SDA, we believe the reviewer has a fundamental misconception about the objective of the SDA method and similar methods to assess risk of vitamin A deficiency in populations. It is quite well known and appreciated that the SDA method or any other dietary method does not accurately indicate the vitamin A status of an *individual*. However,

DIETARY FAT AND INFECTION:
RELATIONSHIP TO VITAMIN A
STATUS OF WOMEN AND THEIR
INFANTS, BREAST MILK RETINOL/
CAROTENOIDS, AND DIETARY
INTAKE METHODOLOGY -

GEORGE
FUCHS

1995 - 011 ✓

these tools are intended to categorize the risk of *groups* of individuals, eg. communities, subpopulations, etc. We are unaware of previous validation of dietary assessment methodologies in pregnant women. This is one reason that this is a principal objective of the proposed study, i.e. to determine the validity in this vulnerable subgroup. Again, since the objective is to test the validity of the SDA method in categorizing a group of pregnant women rather than an individual pregnant woman, we believe this is an entirely appropriate use of the SDA.

It is interesting that the reviewer suggests we should concentrate on lactating rather than pregnant women. However, the same concerns registered by the reviewer for pregnant women with regards to normal variation and vitamin A metabolism are also relevant to lactating women. Even so, the inference that the two groups should be handled differently might have merit. We will therefore stratify these groups in addition to treating them as a single group in the analysis.

Reviewer #2

1. The review appears to suggest the objectives of the study be limited to assessment of the effect of fat on vitamin A bioavailability and status. Clearly, this is the primary objective. However, we believe that the study presents a valuable opportunity to incorporate the important secondary objectives (morbidity assessment and validation of dietary assessment methodologies). It is our opinion that incorporation of these particular secondary objectives will not interfere with attainment of the primary objectives.
2. A principal objective of the study is to enhance bioavailability of carotenoids and provitamin A, not to supplement the diet with these substances. This is particularly important from a programmatic standpoint.