Attachment 1

Date: 6/12/1998

(FACE SHEET) **ETHICAL REVIEW COMMITTEE, ICDDR,B.**

Principal Investigator: Kenneth H Brown & K M A Jamil

Application No. 98-038

Title of Study: Assessment of carotenoid bioavailability from plant sources

Trainee Investigator (if any): _____

Supporting Agency (if Non-ICDDR,B) USDA

Project Status: _____

[x] New Study

[ ] Continuation with change

[ ] No change (do not fill out rest of the form)

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

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

2. Does the Study Involve:
   - (a) Physical risk to the subjects — No
   - (b) Social risk — No
   - (c) Psychological risks to subjects — No
   - (d) Discomfort to subjects — Yes
   - (e) Invasion of privacy — No
   - (f) Disclosure of information damaging to subject or others — No

3. Does the Study Involve:
   - (a) Use of records (hospital. medical, death or other) — No
   - (b) Use of fetal tissue or abortus — No
   - (c) Use of body fluids — Yes

4. Are Subjects Clearly Informed About:
   - (a) Nature and purposes of the study — Yes
   - (b) Procedures to be followed including alternatives used — Yes
   - (c) Physical risk — Yes
   - (d) Sensitive questions — Yes
   - (e) Benefits to be derived — Yes
   - (f) Right to refuse to participate or to withdraw from study — Yes
   - (g) Confidential handling of data — Yes
   - (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — Yes

5. Will Signed Consent Form be Required:
   - (a) From subjects — Yes
   - (b) From parents or guardian (if subjects are minor) — No

6. Will precautions be taken to protect anonymity of subjects — Yes

7. Check documents being submitted herewith to Committee:
   - ___ Umbrella proposal - Initially submit an with 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
   - NA Informed consent form for parent or guardian
   - ✓ Procedure for maintaining confidentiality
   - NA 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. Example 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 Committee 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.

Kazi Mohammad Asif Jamil

Principal Investigator

Trainee

International Centre for Diarrhoeal Disease Research, Bangladesh

RESEARCH PROTOCOL

FOR OFFICE USE ONLY
Protocol No: Date:
ERCApproval: Yes/ No Date:
ERC Approval: Yes/No Date:

1. Title of Project (Do not exceed 60 characters including spaces and punctuations)
Assessment of carotenoid bioavailability from plant sources

2a. Name of the Principal Investigator(s) (Last, First, Middle).	2b. Position / Title	2c. Qualifications
PI # 1: Brown, Kenneth H	Professor	MD
PI # 2: Jamil, Kazi Mohammad Asif	Senior Medical Officer	MBBS, PhD

3. Name of the Division/ Branch / Programme of ICDDR,B under which the study will be carried out.
Clinical Sciences Division

4. **Contact Address of the Principal Investigators**

4a. Office Location: PI # 1: Department of Nutrition, University of California, Davis, CA 95616-8669, USA
PI # 2: Clinical Sciences Division, ICDDR,B, Mohakhali, Dhaka 1212, Bangladesh

4b. Fax No:	4c. E-mail:	4d. Phone / Ext:
PI # 1: 1(916)752-3406	PI # 1: khbrown@ucdavis.edu	PI # 1: 1(916)752-1992
PI # 2: 880-2-9885657	PI # 2: jamil@icddrb.org	PI # 2: 880-2-871751/60 Ext 2314

5. Use of Human Subjects	5a. Use of Live Animal	5b. If Yes, Specify Animal Species
Yes [X]	Yes [ ]	
No [ ]	No [X]	

6. Dates of Proposed Period of Support *(Day, Month, Year - DD/MM/YY)*	7. Cost Required for the Budget Period
01/01/1999 – 31/12/2001	*7a. Ist Year ($): 114,358* *2nd Year ($): 82,998* *3rd Year($): 19,303*
	7b. Direct Cost ($) 216,659 **Total Cost ($) 270,824**

8. Approval of the Project by the Division Director of the Applicant

The above-mentioned project has been discussed and reviewed at the Division level as well by the external reviewers. The protocol has been revised according to the reviewer's comments and is approved.

George J. Fuchs, MD
Name of the Division Director

Signature

6/12/98
Date of Approval

9. Certification by the Principal Investigator

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

10. Signature of PI

Kazi Mohammad Asif Jamil

Date: 6/12/98

Table of Contents

☐ Check here if appendix is included

PROJECT SUMMARY: Describe in concise terms, the hypothesis, objectives, and the relevant background of the project. Describe concisely the experimental design and research methods for achieving the objectives. This description will serve as a succinct and precise and accurate description of the proposed research is required. This summary must be understandable and interpretable when removed from the main application. **(TYPE TEXT WITHIN THE SPACE PROVIDED).**

Principal Investigators: PI#1 Kenneth H Brown
PI#2 Kazi Mohammad Asif Jamil

Project Name: Assessment of carotenoid bioavailability from plant sources

Total Budget: US$ 270,824 Beginning Date: January 1999 Ending Date: December 2001

Recent studies cast doubt on the factors used to convert dietary provitamin A to equivalent amounts of retinol. We therefore plan to conduct human volunteer studies to assess vitamin A bioavailability from different dietary sources, using the novel deuterated-retinol dilution technique to measure change in vitamin A reserves before and after a 60 day period of daily supplementation of a very low vitamin A-containing basal diet with presumably equivalent amounts (1.5mg RE) of either retinol, β-carotene, spinach, or (corn oil) placebo. The conversion of β-carotene to retinol will be assumed to occur at a ratio of 6:1, as is currently recommended by the Food and Nutrition Board. Vitamin A pool size will be measured at baseline using oral tetra-deuterated retinyl acetate (d-4R) and analysis of plasma retinol (R) isotopic ratios (d-4R/R) 21 days later by GC-MS following isolation of retinol by HPLC. The supplements will then be provided for 60 days, and following a 10 day period of stabilization, the isotope dilution studies will be repeated. The difference in pre- and post-supplementation vitamin A pool size will be used to measure the amount of vitamin A retained from each dietary source. Additionally, a second isotope (octa-deuterated retinyl acetate, d-8R) will be administered on the first day of the dietary supplementation period to assess whether the plasma d-8R/R isotopic ratios measured 5, 7, or 10 days after the dose can successfully predict the change in vitamin A pool size in relation to dietary supplements. This would permit a more rapid, simplified assessment of carotenoid bioavailability. The studies will be carried out in Dhaka, Bangladesh, where our previous research indicates that the subjects have low vitamin A reserves and predictable changes in vitamin A pool size in response to different levels of vitamin A (retinol) intake.

KEY PERSONNEL (List names of all investigators including PI and their respective specialties)

Name	Professional Discipline/ Specialty	Role in the Project
1. Kenneth H Brown	Physician-scientist (Nutrition)	PI
2. Kazi Mohammad Asif Jamil	Physician-scientist (Nephrology)	PI
3. Marjorie J Haskell	Nutrition scientist	Co-PI
4. George J Fuchs	Physician-scientist (Pediatrics)	Co-PI

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be tested:

Concisely list in order, in the space provided, the hypothesis to be tested and the Specific Aims of the proposed study. Provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

1. The DRD technique will detect changes in total body vitamin A pool size in response to supplementation with different dietary sources of vitamin A, and the magnitude of the change in pool size will be related to the dietary source of vitamin A.

2. A simplified procedure based on plasma isotopic ratios measured 5-10 days after initiating carotenoid-containing diets will predict the change in total body vitamin A pool size in response to different dietary sources of vitamin A.

Specific Aims:

Describe the specific aims of the proposed study. State the specific parameters, biological functions/ rates/ processes that will be assessed by specific methods (TYPE WITHIN LIMITS).

1. To estimate the total body vitamin A pool sizes of adult Bangladeshi volunteers, using the deuterated retinol dilution (DRD) technique at baseline and after a two month period of providing a controlled diet containing a plant or synthetic source of vitamin A (the "paired DRD technique"). Differences in change in pool size by source of vitamin A will be used to assess relative vitamin A bioavailability from each source.

2. To compare the ability of different techniques to estimate the relative absorption and bioconversion of these different vitamin A sources. The specific techniques that will be compared are: 1) the paired DRD technique, 2) change in plasma retinol concentrations and 3) a simplified procedure based on relative plasma retinol isotopic ratios measured 5-10 days after initiating vitamin A or carotenoid-containing diets.

Background of the Project including Preliminary Observations

Describe the relevant background of the proposed study. Discuss the previous related works on the subject by citing specific references. Describe logically how the present hypothesis is supported by the relevant background observations including any preliminary results that may be available. Critically analyze available knowledge in the field of the proposed study and discuss the questions and gaps in the knowledge that need to be fulfilled to achieve the proposed goals. Provide scientific validity of the hypothesis on the basis of background information. If there is no sufficient information on the subject, indicate the need to develop new knowledge. Also include the **significance and rationale** of the proposed work by specifically discussing how these accomplishments will bring benefit to human health in relation to biomedical, social, and environmental perspectives. **(DO NOT EXCEED 5 PAGES, USE CONTINUATION SHEETS).**

Introduction

Vitamin A deficiency is a serious public health problem in more than 90 countries, with an estimated 230 million children at risk of the deficiency and its complications (1). Severe vitamin A deficiency continues to be the single most important cause of childhood blindness, and marginal deficiency has been associated with increased mortality from common childhood diseases (2, 3). Supplementation with vitamin A has been shown to significantly reduce mortality by ~23% among preschool children (4). Pregnant and lactating women whose diets are habitually low in vitamin A-rich foods are also at risk of vitamin A deficiency (1), and supplementation shortly after birth has been shown to improve maternal serum and breastmilk retinol concentrations (5). Moreover, in a recent longitudinal, placebo controlled trial in Nepal, supplementation of women of reproductive age with a capsule containing 7 mg RE weekly, either as preformed vitamin A or ß-carotene resulted in a 38% reduction in maternal mortality in the vitamin A group and a 50% reduction in the ß-carotene group (6).

One of the long-term strategies for improving vitamin A status is to increase dietary vitamin A intake among populations at risk of deficiency. In developing countries, it is estimated that 65-85% of vitamin A in the diet is supplied by provitamin A-rich vegetables and fruits (7). Although animal products can provide preformed retinol, which is readily available for absorption, these foods are generally not affordable for the populations at greatest risk of deficiency (7). For this reason, nutrition education programs and home garden projects have been implemented in many countries to promote the consumption and cultivation of local vegetables and fruits that are rich sources of provitamin A carotenoids (8). It has been assumed that increased consumption of these foods will result in improved vitamin A status. However, recent evidence suggests that provitamin A carotenoids from plant foods may not be as bioavailable as previously assumed (9, 10). Thus, it is difficult to estimate the amount of provitamin A carotenoids needed from plant foods to satisfy dietary vitamin A requirements. This information is important for developing strategies to improve vitamin A status among populations in developing countries, and for quantifying more accurately the contribution of provitamin A carotenoids from plant foods to vitamin A intake among vegans and populations consuming mixed diets in the U.S. and other industrialized countries.

Among populations in industrialized countries, carotenoids appear to be protective against chronic and degenerative diseases. Studies have indicated that dietary carotenoids are associated with a lower of risk of cancer (11), cardiovascular disease (12, 13), macular degeneration (14), and cataracts (15). However, recently it was reported that long-term supplementation with ß-carotene had no effect on the incidence of malignant neoplasms and cardiovascular disease (16), and supplementation with high-doses of ß-carotene had an adverse effect on the incidence of lung cancer among smokers (17). High doses of synthetic carotenoids may not be optimal for protecting against certain diseases. Provision of smaller doses of carotenoids from natural food sources may be a more effective way to promote health. It has been suggested that the protective effects of carotenoids may be related to improved immune function and antioxidant protection (18). It is also possible that retinoids derived from provitamin A carotenoids are important in protecting against disease. Thus, information on the relative bioavailability of carotenoids from plant foods is needed to determine which foods are good sources of dietary carotenoids and vitamin A.

Vitamin A equivalency of provitamin A carotenoids. Provitamin A carotenoids are predominantly found in dark green leafy vegetables, and certain dark yellow and orange fruits and vegetables. Although, more than 600 carotenoids have been identified in nature, it is estimated that only about 10% of these have vitamin A activity. For a carotenoid to have vitamin A activity it must have at least one intact ß-ionone ring and an isoprene side chain of at least 11 carbon units. Of the provitamin A carotenoids that have been identified, only ß-carotene, α-carotene, γ-carotene, and ß-cryptoxanthin are commonly found in the diets of humans. Of these four carotenoids, ß-carotene has the highest vitamin A activity. The others have approximately half of the vitamin A activity of ß-carotene. In theory, ß-carotene can be enzymatically

cleaved in the intestine to yield two molecules of vitamin A because it has two intact ß-ionone rings and isoprene side chains (19). However, the bioconversion of provitamin A carotenoids in the intestine has been shown to be less efficient (20, 21). The bioconversion factors that are currently recommended by the Food and Nutrition Board to estimate the vitamin A activity of carotene-rich foods are based on the assumptions that approximately 33% of dietary provitamin A carotenoids is absorbed, and about 50% of absorbed ß-carotene is converted to vitamin A in the human intestine (22). Thus, the factor that is used for the conversion of ß-carotene to vitamin A is 6:1, whereas the conversion factor for the other provitamin A carotenoids is 12:1 because they have approximately 50% of the vitamin A activity of ß-carotene. Thus, 6 µg of ß-carotene is assumed to be equivalent to 1 µg of retinol or 1 µg retinol equivalent (RE), whereas 12 µg of the other provitamin A carotenoids is assumed to be equal to 1 µg RE.

Absorption of carotenoids. Carotenoids are found primarily in protein complexes in plant foods. When these foods are consumed, carotenoids are released from the protein complexes in the stomach, and aggregate with other dietary lipids to form lipid globules. In the intestinal lumen these globules are incorporated into mixed lipid micelles. Carotenoids are taken up into the intestinal mucosal cells by passive diffusion. The efficiency of absorption has been reported to range from 2 - 50% (23). Within the intestinal cell, carotenoids can be incorporated intact into chylomicrons, enzymatically cleaved in the central position to yield retinal, or excentrically cleaved to yield ß-apo-carotenals (24). The retinal that is produced can be reduced to retinol and absorbed as preformed vitamin A as described above. The ß-apo-carotenals can also be further metabolized to retinal, reduced to retinol and absorbed as vitamin A, or esterified and incorporated into chylomicrons as ß-apo-carotenal esters. Alternatively, small amounts of retinal and ß-apo-carotenals may be oxidized to form retinoic acid, ß-apo-carotenoic acids or other polar metabolites, which can be absorbed into the circulation through the portal vein (25).

Factors affecting carotenoid absorption. Absorption of carotenoids is affected by dietary factors, food preparation and processing techniques, the chemical form in which carotenoids are found in foods, as well as host-related factors. Dietary fat and vitamin E enhance the absorption of carotenoids (23), whereas dietary fiber impairs carotenoid absorption (26). Dietary fat stimulates the production and release of bile salts, which are necessary for formation of micelles in the intestinal lumen. Vitamin E acts as an antioxidant and protects carotenoids from oxidation within the intestinal lumen. In contrast, dietary fiber, particularly citrus pectin, significantly reduces the absorption of ß-carotene (26), probably through binding bile salts and interfering with the formation of micelles within the intestinal lumen.

The way in which carotenoids are stored in plant tissue may also affect their bioavailability. In green photosynthetic plant tissue, carotenoids are found in chloroplasts in pigment-protein complexes. In non-photosynthetic plant tissue, carotenoids are found in small lipid droplets in chromoplasts. There is speculation that because carotenoids in chromoplasts are stored in lipid droplets they may be more bioavailable than carotenoids in chloroplasts (23, 27).

The method with which food is prepared also affects the bioavailability of carotenoids. For example, particle size of foods and the cooking process have been reported to affect carotenoid absorption (28). Carotenoids in finely chopped, or pureed raw foods appear to be better absorbed than sliced or whole raw foods (21). Mild heating enhances absorption of carotenoids, probably through denaturing protein and thereby releasing carotenoids from the protein complex (29). However, excessive heating can result in the formation of 9 or 13 cis isomers, which may not be well absorbed (30). Similarly, oxidation of carotenoids during cooking or processing will also result in lower bioavailability (23).

Host factors that influence lipid absorption in general also affect uptake of carotenoids. For example, gastrointestinal disease, diarrhea and intestinal parasites decrease the absorption of carotenoids (7, 23, 31). It has also been suggested that low vitamin A status may enhance the bioconversion of provitamin A carotenoids to vitamin A (32). Because all of these factors may affect the utilization of carotenoids, it is very difficult to predict the bioavailability of carotenoids from foods.

Absorption of carotenoids in human subjects. Several studies have examined absorption of ß-carotene by monitoring changes in plasma ß-carotene levels following consumption of carotene-containing meals or synthetic supplements. Although these studies may provide some insight into relative efficiency of absorption, they are difficult to interpret because they do not distinguish between the ß-carotene that is absorbed and removed from the circulation for storage in tissue, or converted to retinol, and the ß-carotene that is simply excreted in the feces. Nevertheless, the bulk of evidence from these studies suggests that ß-carotene from food sources is less well absorbed than synthetic ß-carotene, and that the absorption of carotenoids is highly variable among different individuals.

Brown et al (10) fed adult men a single dose of 30 mg or 12 mg of ß-carotene in oil or a similar amount of ß-carotene from vegetable sources in meals that contained 40% of energy as fat in a crossover design. They found that the increase in the plasma ß-carotene concentration in response to carrots was onl about 14% of the response observed for an equivalent amount of synthetic ß-carotene, and that no significa increase in the plasma ß-carotene concentration was observed in response to supplementation with ~6 mg c ß-carotene in broccoli. They also reported that the plasma response to synthetic ß-carotene varied 3 to 4-fc in healthy adult men (10). Similarly, Micozzi et al (33) found that the increase in the plasma ß-carotene concentration in response to carrots was only about 18% of the response observed for a similar amount of f carotene in oil, and no change in plasma ß-carotene concentration was observed in response to broccoli. Likewise, Bulux et al (32) reported that the concentration of ß-carotene in plasma increased significantly in Guatemalan children who were supplemented with synthetic ß-carotene for 20 days, but not in children wh received a similar amount of ß-carotene from cooked carrots. de Pee et al (9) reported a significant increas in the plasma ß-carotene concentration among lactating women who were supplemented for 12 weeks with an enriched wafer containing 3.5 mg ß-carotene, but only a slight increase among lactating women who received an equivalent amount of ß-carotene from dark green leafy vegetables or carrots. More recently, dc Pee et al (34) reported results of a second study in which they observed a significant increase in the plasma ß-carotene concentration among Indonesian schoolchildren who received two

servings of mango and papaya daily over a period of 9-weeks, but a significantly lower increase among children who received an equivalent amount of ß-carotene from dark green leafy vegetables or carrots.

The results of these studies indicate that ß-carotene from foods is less well absorbed than synthetic f carotene in a simple matrix, such as an enriched wafer or dissolved in oil. Moreover, these results suggest that the assumption that 33% of dietary carotenoids is absorbed in the human intestine needs to be re-examined. However, as stated above, these results are difficult to interpret because plasma carotenoid concentrations do not reflect bioconversion of provitamin A carotenoids to vitamin A, or carotenoids that were removed from the plasma and stored in tissue. Thus, the quantitative relationship between carotenoid intake and bioconversion to vitamin A remains uncertain.

Bioconversion of provitamin A carotenoids in human subjects. Because provitamin A carotenoids can be converted to retinal in the human intestine, it is important to examine the bioconversion of these carotenoids to vitamin A in response to supplementation when assessing bioavailability. Few well-controlled studies have looked at the effect of dietary supplementation with provitamin A carotenoids on the vitamin A status of individuals. Bulux et al (32) reported no change in serum retinol levels among Guatemalan children who were supplemented with synthetic ß-carotene or a similar quantity of ß-carotene from cooked carrots. However, these children were vitamin A replete, and serum retinol concentrations would not be expected to increase in response to supplementation in replete individuals. de Pee et al (9) reported a significant increase in serum and breastmilk retinol concentrations among anemic, lactating Indonesian women who received a wafer fortified with ß-carotene, but no change among women who received an equivalent amount of ß-carotene from stir-fried spinach or carrots. The fat content of the supplements was similar. It is likely that this group of women was marginally deficient, because a significant response in serum and breastmilk retinol concentrations was observed in the group that received the enriched wafer. However, women were assigned to the vegetable or wafer treatment groups by village rather than by individual; thus, differences in the baseline vitamin A status of women in the two villages cannot be ruled out. In their more recent study, de Pee et al (34) reported a significant increase in plasma retinol concentration among Indonesian schoolchildren who received two servings of mango and papaya daily, but no response in children who received an equivalent amount of ß-carotene from dark green leafy vegetables or carrots. The mean increase in the plasma retinol concentration among children who received fruit, was approximately two-thirds of the mean increase observed for children who received an equivalent amount of vitamin A as preformed retinol from animal foods such as liver and egg yolk. The 6:1 conversion ratio for ß-carotene:retinol was used to provide equivalent amounts of vitamin A as ß-carotene from food sources, and the fat content of the meals was similar. Thus, it appears that provitamin A carotenoids from papaya and mango may be more bioavailable than provitamin A carotenoids from dark green leafy vegetables or carrots. Jalal et al (31) also reported an increase in serum retinol concentrations among Indonesian children who received a vegetable and fat supplement daily for 24 days. The vegetable supplement consisted of a mixture of mostly red sweet potatoes and dark green leafy vegetables. It is possible that the provitamin A carotenoids in fruit and sweet potato are more bioavailable because they are found in chromoplasts rather than chloroplasts, as described earlier. However, it is difficult to make quantitative interpretations on relative bioavailability based on serum retinol concentrations because of uncertainties regarding factors influencing the storage and circulation of vitamin A.

Assessment of bioconversion in human subjects. The absorption and bioconversion of provitamin A carotenoids to vitamin A in humans is difficult to study because commonly used vitamin A assessment techniques do not provide a quantitative estimate of total body stores of vitamin A. Plasma retinol levels are homeostatically maintained, and are not likely to change in response to supplementation with provitamin A carotenoids, unless the subjects are vitamin A deficient at the onset of the intervention. Even when vitamin A levels do respond to supplementation in depleted individuals, the magnitude of increase may not be directly proportional to bioavailability from a particular vitamin A source. Similarly, the relative dose response tests categorize individuals as replete or depleted only, and do not provide a quantitative estimate body stores of vitamin A. Therefore, it is not possible to obtain quantitative information on the relative change in vitamin A body stores in response to supplementation with foods rich in provitamin A carotenoic when these indirect assessment techniques are used.

Deuterated retinol dilution technique. It is estimated that greater than 90% of total body vitamin A stored in the liver in well-nourished individuals (7), therefore the hepatic vitamin A concentration provides quantitative estimate of total body vitamin A stores. Because of the invasiveness of obtaining liver biopsy specimens, this is not a feasible method for routine assessment of vitamin A status. However, the deuterate retinol dilution (DRD) technique has been introduced recently as an indirect assessment technique for quantitatively estimating total body stores of vitamin A in human subjects. Briefly, a known dose of d4-retinyl acetate is administered orally, and after a period of equilibration with the body pool of vitamin A (~ days), a blood sample is drawn for measurement of the isotopic ratio of retinol-d4/retinol in plasma. Hepat reserves of vitamin A are estimated based on the principles of isotope dilution, and a set of assumptions, fir described by Bausch and Rietz (35) and later modified by Furr et al (36):

$$\text{Total liver reserves} = F \times \text{dose} \times (S \times a \times \{(1/D{:}H)-1\})$$

where F is a factor for efficiency of storage of an orally administered dose, which is assumed to be 0.5 base on the previous work of Bausch and Rietz (35); the dose is the amount of isotope administered in milligram retinol equivalents; S corrects for the inequality of the plasma to liver ratio of retinol-d4/retinol; a is used to correct for irreversible loss of labeled vitamin A during the

equilibration period; the D:H ratio is the isotopic ratio of retinol-d4/retinol in plasma, and finally, the value 1 corrects for the contribution of the dose to hepatic stores of vitamin A. For the factor S, a value of 0.65 is used, which is based on the mean observed plasma to liver ratio of the specific activities of radiolabeled vitamin A in rats with varying body stores of vitamin A (37). The factor a is based on the half-life of vitam A turnover in the liver, which is estimated as 140 days, and is assumed to be independent of the size of the liver reserves of vitamin A, and time-invariant ($a=e^{-kt}$, where k=1/140d and t=time in days since dose) (20, 36, 38).

Validation of the DRD technique. The DRD technique has been partially validated against the hepatic vitamin A concentration in a small group of well-nourished surgical patients in the US (36), and we have further validated the technique in a group of surgical patients in Bangladesh who had low body stores of vitamin A (39). As shown in Table 1, the DRD technique provided a very good quantitative estimate of total body stores of vitamin A for the group of Bangladeshi patients, using a single plasma isotopic ratio measurement which was obtained 18-25 days post-dose. Our recent data on plasma retinol kinetics in response to an oral dose of d4-retinyl acetate in adult Bangladeshi and North American subjects (Figure 1) indicate that the test dose of labeled vitamin A mixes with endogenous body stores of vitamin A by 20 days in subjects with relatively low or high body stores of vitamin A, as estimated by the DRD technique (40). Thus, this indicates that the 20 d time point is suitable for estimating total body stores of vitamin A using th method of Furr et. al. (36).

Application of the DRD technique. Our results from a more recent study conducted in Bangladesh indicate that the DRD technique can be used to detect changes in total body stores of vitamin A in response to supplementation (41). Briefly, adult men were kept on a basal diet low in vitamin A for 128 days and were supplemented with either 0, 1.5 or 3.0 mg RE of retinyl palmitate in corn oil daily during the 128-d period. Two dilution studies were conducted using the DRD technique. The first dose of isotope was administered on day 1, and daily supplementation with the different levels of unlabeled vitamin A was started on day 2 and continued for 128 days. Blood was collected at 18 time points over a 90-day period for measurement of the isotopic ratio of retinol-d4/retinol to generate plasma kinetic curves. The purpose of beginning supplementation with unlabeled vitamin A during the initial kinetic time course was to assess the effect of unlabeled dietary vitamin A on the plasma isotopic ratio of retinol-d4/retinol during the period of isotopic "equilibration". The second dose of isotope was administered on day 90 to estimate total body stores of vitamin A following 90 days of supplementation. Blood was drawn at four time points over a 37

day period (day 90-127) following the second dose of isotope, and the plasma isotopic ratio on day 115 (25 days after the second dose of isotope was administered) was used to estimate total body stores of vitamin A. Highly significant differences in the mean vitamin A pool sizes were observed across the three treatment groups after the 90-d supplementation period ($p<0.001$, Table 2). Moreover, the estimated mean change in vitamin A body stores in the two supplemented groups was similar to the expected theoretical increase whe compared to the control group, assuming that 50% of the supplemental vitamin A was retained (35) and 0.5%/d was catabolized (38) (Table 3). Thus, the DRD technique detected increases in total body stores c vitamin A in response to the different levels of supplementation that were similar to the expected theoretic increases. Therefore, this technique could also be used to measure changes in pool size from the beginning to the end of a period of time in which different food sources of carotenoids provide the major source of vitamin A. Differences in the change in vitamin A pool size across dietary treatment groups would indicat the absorption and bioconversion of carotenoids from these food sources.

The results from this study also indicate that the plasma isotopic ratios of retinol-d4/retinol are sensitive to dietary vitamin A intake. Therefore, it may be possible to assess the bioavailability of carotenoids from foods rapidly, by estimating the extent to which labeled vitamin A is diluted in the plasm by recently consumed non-labeled dietary vitamin A that is derived from provitamin A carotenoids. As mentioned above, an oral dose of labeled vitamin A does not truly equilibrate with endogenous body stores of vitamin A because of the continuous intake of unlabeled dietary vitamin A during the mixing period. Because newly absorbed vitamin A is preferentially secreted into the plasma pool from the liver as RBP-retinol (42), unlabeled dietary vitamin A will affect the plasma isotopic ratio of retinol-d4/retinol. Our results indicate that the dilution of labeled vitamin A in the plasma pool is related to the amount of non-labeled vitamin A consumed in the diet. As shown in Figure 2, in our previous study the mean plasma isotopic ratios were similar across treatment groups on day 1, but significantly different by day 4 and thereafter. Total body stores of vitamin A were estimated for the three groups of subjects using the isotopic ratios on day 20 and the model described by Furr et al (36). However, the estimated mean change in total body stores of vitamin A in the supplemented groups when compared with the control group was higher tha the expected theoretical increase based on the assumptions that 50% of the supplemental vitamin A was retained (35), and that the fractional catabolic rate was ~0.5%/d (38), (20) (Table 4). The probable explanation for this discrepancy is that newly absorbed unlabeled vitamin A provided by the daily supplement was preferentially secreted into the plasma. For this reason, the labeled vitamin A in the plasm pool would be more highly diluted than the labeled vitamin A in the liver pool. A lower plasma isotopic ratio of retinol-d4/retinol would result in an overestimate of total body stores of vitamin A, as was observed However, as described above, the estimates of the mean increase in total body stores of vitamin A for the supplemented groups were similar to the theoretical estimates of the expected increase when the second dilution study was conducted following 90 days of supplementation (Table 3). During the 90-day supplementation period, the subjects had adjusted to their assigned level of vitamin A intake. Thus, the dail dose of unlabeled vitamin A or corn oil during the second "equilibration" period no longer interfered with tl estimate of total body stores of vitamin A. Therefore, under carefully controlled conditions in which all of the dietary vitamin A is derived from provitamin A carotenoids, it should be possible to assess the relative bioavailability of provitamin A carotenoids from foods by comparing the extent to which labeled vitamin A is diluted in the plasma pool within a short period of time (~5-10d) after initiating daily supplementation with provitamin A-rich foods, preformed vitamin A or synthetic ß-carotene.

Rationale and significance. The proposed application of the DRD technique is a novel, quantitative method for estimating the bioavailability of dietary provitamin A carotenoids in human subjects. The relative bioavailability of provitamin A carotenoids from different foods can be measured by comparing the change in body stores of vitamin A in response to supplementation with food sources of ß-carotene or an equivalent amount of vitamin A as purified ß-carotene. Furthermore, vitamin A equivalency factors can be defined by comparing the change in total body stores of vitamin A that occurs in response to ß-carotene or carotene-containing foods with that which occurs in response to supplementation with a presumably equivalent amount of vitamin A as retinyl palmitate. This information is needed to reassess currently used conversion factors to estimate the vitamin A contribution of provitamin A carotenoids from different plant foods.

The purpose of the study proposed herein is to use the DRD technique to assess the bioavailability o provitamin A carotenoids from different dietary sources, specifically a green leafy vegetable and from purified ß-carotene. Initially, a set of detailed studies will be conducted under carefully-controlled clinical conditions to assess the change in total body stores of vitamin A in response to supplementation with these provitamin A carotenoids. At the same time the possible use of an abbreviated assessment technique will b evaluated. Plasma carotenoid profiles will also be compared before and after the supplementation period to assess the absorption of intact carotenoids. We will simultaneously attempt to identify additional sources of

funding to study other plant sources (sweet potato) of provitamin A carotenoids. However, the interpretability of the present study is not dependent on obtaining that additional funding. Finally, after refining the abbreviated assessment technique based on the results of these initial studies, subsequent proposals will be developed using the simplified technique to examine different dietary sources of carotenoids and the effects of different levels of dietary fat and fiber, and food preparation techniques on th relative bioavailability of provitamin A carotenoids from foods. Eventually, this set of information should useful to develop algorithims for the conversion of dietary provitamin A carotenoids to vitamin A equivalents under different conditions.

Research Design and Methods

Describe in detail the methods and procedures that will be used to accomplish the objectives and specific aims of the proje Discuss the alternative methods that are available and justify the use of the method proposed in the study. Justify the scienti validity of the methodological approach (biomedical, social, or environmental) as an investigation tool to achieve the speci aims. Discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them. Discuss t ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in si children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Point out saf procedures to be observed for protection of individuals during any situations or materials that may be injurious to human heal The methodology section should be sufficiently descriptive to allow the reviewers to make valid and unambiguous assessment the project. **(DO NOT EXCEED TEN PAGES, USE CONTINUATION SHEETS).**

Study site. The study will be conducted at the previously established volunteer ward of the International Centre for Diarrhoeal Disease Research in Dhaka, Bangladesh. This site has been chosen because former studies have indicated that vitamin A depletion is common in Bangladesh, thus making it easier to recruit subjects with low vitamin A reserves and to detect changes in vitamin A reserves in respon to dietary supplements. Moreover, because local diets are typically very limited in vitamin A content, it is possible to provide baseline study diets having low vitamin A contents without imposing hardship on the volunteer subjects. In addition to the research ward, the study center has kitchen facilities for the preparatic of carefully defined study diets and laboratory facilities for the analysis of clinical specimens.

Subjects. The subjects will be non-ill adult males from 18-35 years of age. We have decided to wo with adult males, as in our previous volunteer study, because we could not hospitalize children for this leng of time and because it would be extremely difficult to recruit women for the proposed duration of the study in the conservative Muslim culture of Bangladesh. Entry criteria will include: low plasma retinol concentration (<1.05 μmol/L), but no clinical evidence of vitamin A deficiency; adequate protein status (serum albumin >35 g/L); and no evidence of intestinal malabsorption or other conditions that may interfere with vitamin A absorption or metabolism. Stool samples will be examined microscopically and parasitic infections will be treated as necessary. Subjects with anemia will be screened for iron deficiency and treate if appropriate. Our previous studies indicate that subjects fulfilling these entry criteria will have low total body vitamin A pool sizes (average ~ 12 mg, as determined by the DRD technique). Subjects will be examined by a physician weekly. It is unlikely that subjects in the control group will develop symptoms of vitamin A deficiency because although the diet will be low in vitamin A, they will receive labeled vitamin A at various times throughout the study period as described below. However, if any subject develops clinical symptoms of vitamin A deficiency, he will be treated with vitamin A. The study protocol has been approve by the Institutional Review Board at the University of California-Davis (Appendix 4) and will be submitted to the corresponding committee at ICDDR,B. The clinical procedures are very similar to our previous study which was approved by both of these human subjects oversight committees.

"Paired DRD-technique". Once the subjects complete the initial screening procedures and receive any necessary therapy, they will begin receiving all three meals per day on the study ward. The volunteers will report to the facility by 7:00 each morning and remain under supervision until 7:00 pm, when they will be permitted to return home for the night. The baseline diet will be composed of rice, wheat flat bread, lentils, small amounts of curried meat, vegetables such as cauliflower, cabbage, and white squash, and fruits such as banana, all of which are very low in vitamin A content. As shown in the graphic summary of the protocol (Figure 3), subjects will consume the low-vitamin A diet for a period of 6 days before receiving the first dose of isotope. A baseline blood sample will be drawn on the morning of day 7, and then the subjects will receive 10 mg of d4-retinyl acetate by mouth along with a high-fat breakfast. Blood will be drawn at 2 hours to assess uptake of the labeled dose. Additional blood samples will be drawn 20 and 21 days after the

administration of labeled vitamin A (protocol days 27 and 28) to estimate the total body vitamin A pool siz as described by Furr and colleagues. We will be drawing blood on two consecutive days so that we can obtain at least two measurements of the plasma isotopic ratio for each subject. The mean isotopic ratio measurement from the two samples will be used to estimate the vitamin A pool size. If any subject develo a febrile illness during the period between administration of the dose of labeled vitamin A and measuremer of pool size 21 days later, he will be dropped from the study.

After the initial assessment of the total body vitamin A pool size the subjects will be ranked according to their initial plasma retinol level and randomly assigned in blocks of four to one of the four treatment groups. Subjects assigned to group 1 will receive two capsules per day containing 0.75 mg RE (total = 1.5 mg RE/d or 1.5 times the RDA) as retinyl palmitate in 200 µL corn oil. A capsule will be given with the noontime and evening meals. Subjects assigned to group 2 will receive two capsules containing th same amount of vitamin A as purified ß-carotene in 200 µL oil (i.e. 4.5 mg ß-carotene/capsule; total of 9 m ß-carotene/d), assuming that 6 RE ß-carotene is the same as 1 RE from retinyl palmitate. Those assigned t group 3 will receive the same amount of vitamin A as cooked spinach with the noontime and evening meal (i.e., 83 g/ meal; total of approximately 165 g spinach, containing 9 mg all-trans ß-carotene), as well as a capsule containing 200 µL corn oil with each meal. Subjects assigned to group 4 will receive two capsules containing 200 µL corn oil only. In all cases the vitamin A sources will be provided with the noontime and evening meals, each of which will contain approximately 13.6 g fat and 29 g fiber.

The specific food item has been chosen because it is an important source of carotenoids in Bangladesh. Moreover, ß-carotene provides all of the provitamin A in spinach (43). Retinyl palmitate (grou 1) will serve as a positive control to indicate the level of change in pool size that occurs when vitamin A availability does not depend on absorption or bioconversion of carotenoid sources. According to our previous studies in similar subjects, we expect that this amount of retinyl palmitate will result in a change ir pool size of ~ 33 mg. Purified ß-carotene in oil (group 2) will serve as an indicator of the level of change ir vitamin A pool size that occurs when carotenoid bioavailability is not influenced by its food matrix. This will also permit a reassessment of the current estimate of the conversion factor of ß-carotene:retinol. Spinach will be used to assess the impact of the food matrix on the bioconversion of ß-carotene from green leafy vegetables in which carotenoids are found in chloroplasts. A control group not receiving additional sources of vitamin A (group 4) will be included to assess the change in pool size attributable to the basal die

After blood samples have been drawn for the initial pool size estimate, the baseline diets will be supplemented with the respective vitamin A sources for the next 60 days. As mentioned above, our previou studies indicate that a 60-d supplementation period should provide sufficient time for vitamin A pool sizes t increase by approximately 33 mg, if the predicted level of vitamin A is available from the particular source. At the beginning of the 60-d supplementation period (protocol day 29), subjects will receive an oral dose of mg d8-retinyl acetate. d8-retinyl acetate will be used so that it can be distinguished from the previously administered d4-retinyl acetate. Blood will be drawn 24 hours after administration of the isotope to assess uptake of the dose, and again on days 5, 7, and 10 for measurement of the plasma isotopic ratio of retinol-d8/retinol. Results of these samples will be compared with the increments in pool size in response to the dietary treatments to determine whether relative bioavailability of vitamin A can be predicted from these early plasma isotopic ratios during the supplementation period instead of from the longer duration paired-DRD studies. We have chosen these time points because we have observed in our previous studies that the plasma isotopic ratios among subjects receiving different levels of unlabeled vitamin A daily are very different early in the kinetic time course (day 4) and tend to become more similar thereafter. Thus, it may b possible to detect differences in plasma isotopic ratios among the dietary treatment groups on days 5, 7, or 10. At the end of the supplementation period, (i.e., protocol day 89, a blood sample will be drawn for measurement of plasma carotenoid and retinol concentrations. Beginning on day 90, the subjects will resume the basal, non-supplemented diet for a 10-d stabilization period. The reason for the stabilization period is described below.

In the first round of our previous studies, subjects were supplemented for 75-d, and plasma isotopic ratios were measured over a period of 128-d. These data demonstrated that the plasma isotopic ratio of retinol-d4/retinol increased when supplementation with unlabeled dietary vitamin A was discontinued on da 75. Presumably this occurs because plasma vitamin A is derived from body stores to a greater extent when dietary vitamin A intake is limited. As previously described, newly absorbed dietary vitamin A is preferentially secreted into the plasma pool (42); thus, when dietary intake of unlabeled vitamin A is restricted, there is less of a direct dilution effect on the plasma vitamin A pool. In the first round of our previous study, this "rebound effect" of the plasma isotopic ratio complicated the estimate of total body stores of vitamin A following the second dose of isotope. It was more difficult to correct the measurement the plasma isotopic ratio following the second dose of isotope for the contribution of isotope from the first dose because of the pertubation caused by withdrawing the unlabeled daily dose of vitamin A on day 75.

The study design was revised so that this was not a problem in the second round of our previous study. However, data from the first round indicate that the transient increase in the plasma isotopic ratio stabilizes ~10d after discontinuing supplementation with unlabeled vitamin A. For this reason, the subjects in the present study will be kept on the basal diet for 10 d before the second estimate of the total body vitamin A pool size will be initiated. Two blood samples will be drawn (days 99 and 100) for measurement of the isotopic ratio of retinol-d4/retinol, prior to administering the second dose of d4-retinyl acetate. These isotopic ratios will be used to correct the measurement of the plasma isotopic ratio of retinol-d4/retinol afte the second dose of d4-retinyl acetate for the contribution of any isotope that is remaining in plasma from th first dose of d4-retinyl acetate.

Subjects will receive the second and final dose of 10 mg d4-retinyl acetate on day 100 to estimate total body stores of vitamin A, and will continue to receive the unsupplemented basal diet during the next 2 days to minimize the effect of dietary vitamin A intake on the estimation of the vitamin A pool size. The basal diet contains ~270 µg RE, which should be sufficient to keep the subjects in balance with respect to vitamin A over the 21-d "equilibration" period. Blood samples will be drawn 24 hours after administration of the labeled dose, and again 20, and 21 days later, as with the first dose. The estimated pool size will be compared with the initial pool size within each treatment group, and the increment in the respective pools sizes will be compared by treatment group.

Assessment of plasma carotenoids. The plasma concentrations of ß-carotene, α-carotene, lycopene, ß-cryptoxanthin and lutein will be measured at the beginning and end of the 60d supplementation period by HPLC, as previously described (44), to assess changes in carotenoid concentrations in response to supplementation with different dietary sources of vitamin A.

Diets. The basal diet will consist of rice, wheat flat bread, lentils, small amounts of curried meat, vegetables such as cauliflower, cabbage, and white squash, and fruits such as banana, all of which are very low in vitamin A content. From our previous studies, we have calculated that the basal diet provided ~270 µg RE/d. During the 60-d intervention period, sources of vitamin A will be given with the noontime and evening meals, as described above. These two meals will be standardized, such that the amounts of energy, protein, fat and fiber are equal across treatment groups (Table 5, (45-47)). The fiber content of individual food items will be analyzed (48), and the diets will be adjusted, if necessary, to equalize the amount of fiber across treatment groups. Subjects will be allowed to consume selected low-vitamin A and low-fat foods, such as wheat flat bread, white fruits and vegetables, ad libitim at the breakfast meal and mid-afternoon snack to allow for differences in individual caloric requirements. They will receive a daily vitamin (excluding vitamin A) and mineral supplement containing the RDA for essential vitamins and minerals.

Spinach will serve as the plant source of ß-carotene, and the other three treatment groups will receiv an equivalent amount of cabbage instead of spinach, as shown in Table 5. The spinach and cabbage will be prepared daily as traditionally consumed in Bangladesh, using standardized recipes. Briefly, the vegetables will be steamed, and then pureed and fried in oil (49). Each serving of spinach or cabbage will contain 13.6 g (1 tbsp.) of oil. The vegetables will be purchased from the same producer throughout the 60-d supplementation period to minimize variation in carotenoid content of the spinach. The carotenoid content of spinach that is cooked, pureed and then fried will be determined by HPLC (30) prior to initiating the study to determine the portion sizes required to supply 9.0 mg (1.5 mg RE) as all-trans ß-carotene. The conventional conversion factor for ß-carotene:retinol of 6:1 will be used to determine retinol equivalents of ß-carotene in the spinach (22). All-trans ß-carotene and lutein will be quantified, as well as 13- and 9-cis isomers of ß-carotene and α-carotene, if present. The vitamin A activity of cooked spinach will be based o its all-trans ß-carotene content. Cooked spinach contains ~5.5 mg ß-carotene/100g (43). Therefore, it is estimated that the supplemented diet will contain ~ 165 g of spinach daily. Duplicate portions of spinach will be prepared weekly throughout the study period and frozen at -70 C until analyzed for their carotenoid content.

Duplicate meals (all foods) will be prepared weekly and analyzed for the vitamin A and carotenoid content (50, 51). Each subject will have his own plate, which will be identified by number and weighed. Portions of spinach, along with the other foods, will be weighed out onto tared plates at the noon and evenir meals. Subjects will be supervised during mealtimes, and will be asked to consume all of the food provided Any leftover food will be weighed to determine the amounts of foods consumed, and these amounts will be recorded by the dietitian after each meal. In our previous experience, leftover food was rare, and very minimal in quantity. As previously described, the groups that do not receive spinach will receive a capsule containing either 0.75 mg RE as retinyl palmitate (a total of 1.5 mg RE), 4.5 mg ß-carotene in oil (a total of 1.5 mg RE), or corn oil (0 mg RE) with their noontime and evening meals.

Sample size. The sample size estimate for hypothesis 1 is based on our desire to be able to detect nutritionally important differences in the change in vitamin A pool size from the beginning to the end of th dietary supplementation period in relation to the dietary source of vitamin A. This estimate requires knowledge of the mean and standard deviation of the change in vitamin A pool size under specified conditions. We are able to estimate the expected change in pool size based on either theoretical considerations or the difference between the final pool size of the supplemented groups and the non-supplemented group in our previous study. However, we do not have information on the SD of the change pool size because, as stated earlier and shown in Table 4, we do not believe our measurements of the baseline vitamin A pool size from our previous studies are reliable for those subjects who were receiving supplemental vitamin A at the time the isotope ratios were being measured. However, the SD of the chang in pool size can be estimated by pooling the observed SDs of the final pool size estimates for each of the supplementation groups and assuming that the correlation between initial and final pool size is at least 0.5. Thus, we have carried out the sample size estimate for the first hypothesis based on the observed difference in final pool size between the group that received 1.5 mg RE/d for 90 days and the unsupplemented group. In our previous study the difference in the final vitamin A pool size for the vitamin A supplemented (1.5 m RE/d) and placebo groups was 58.3 mg which was very similar to the expected theoretical difference (Tabl 3). Based on these data, we expect to observe a change in pool size of 33 mg in response to 60 days of supplementation with 1.5 mg RE of retinyl palmitate/d. If the 6:1 conversion factor for ß-carotene:retinol i correct, we should observe a difference of ~33 mg in the ß-carotene and vitamin A supplemented groups. However, if the conversion factor is 8:1 or higher, we need to be able to detect a difference in pool size of 8.3 mg across treatment groups. This corresponds to the expected difference in pool size between the vitamin A and ß-carotene groups (~33 vs. ~24.7 mg) if the conversion factor is 8:1 rather than 6:1. Thus, assuming that each of the groups will have similar mean pool sizes initially, we want to be able to detect a difference in final pool size of approximately 8.3 mg by dietary group, which is one quarter of the expected change (33.3 mg) in pool size for the group receiving 1.5 mg RE as retinyl palmitate daily for 60 days. To detect this level of difference with alpha=0.05 and 1-beta =0.80, a sample size of 14 per group is needed. We believe this estimate is conservative for two reasons. First, in the present study we will have reliable estimates of baseline and final pool sizes, and we will not have to make any assumption regarding initial similarity of pool size by treatment group. Second, based on recent studies, it is conceivable that the relativ bioavailability of carotenoids from plant sources is even less than the factor of ~8:1 (i.e. ~one fourth different from the currently assumed 6:1) which will be detectable with this sample size.

The second purpose of the study is to assess the feasibility of predicting the change in pool size fron the isotopic ratios measured several days after giving labeled vitamin A along with the dietary sources to be evaluated. A sample size of 14 per group is sufficient to detect a correlation of 0.65 between early isotopic ratios and change in pool size. (If the true correlation is less than 0.65, then early isotopic ratios would hav questionable usefulness as a predictor). To place this correlation coefficient in context, we have examined the correlation between isotopic ratios on days 5, 7, and 10 and the final vitamin A pool size in our previous study. These correlations ranged from 0.93-0.97 on the different days of observation. Thus, we believe tha a sample size of 14 will be adequate.

The final sample will be inflated to 16 per group (total N=64) to allow for attrition, due to either poc subject compliance or intercurrent illness.

Measurements of plasma isotopic enrichment. The isotopic ratio of retinol-d4/retinol in plasma will be determined by GC-MS as first described by Clifford et al (52) and modified by Handelman et al (53). Briefly, retinol will be isolated from plasma by HPLC, and the tert-butyldimethylsilyl (tBDMS) derivative retinol will be formed. Isotopic ratios will be estimated by GC/MS on a Shimadzu QP 5000 quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) using 70 eV electron ionization. Selected ion monitoring will be carried out for fragment ions of the tBDMS derivatives at m/z 255 (retinol) and m/z 259 (retinol-d4). A set of calibration standards with retinol-d4/retinol weight ratios of 0.00, 0.0167, 0.050, 0.167, 0.500 will be analyzed with each set of plasma samples. A linear regression equation will be calculated between the weight ratios of the calibration standards and the integrated areas for m/z 259 and 255. The area ratios for the plasma samples will substituted into the regression equation to solve for the weight ratios. The within-run precision of the isotopic ratio measurements will be estimated by analyzing standards periodically with each set of plasma samples. The coefficient of variation for the mean isotopic ratio measurements for the standards is typically <5%. Plasma isotopic ratios of retinol-d8/retinol will also be measured by GC/MS, as described above, but using fragment ions at m/z 263 and m/z 255.

For our previous USDA-supported study in Bangladesh, we collected ~660 plasma samples for measurement of the plasma isotopic ratio of retinol-d4/retinol that we had intended to analyze over a two-year period. We had difficulties completing those analyses on time because we were dependent upon a gas

chromatograph/mass spectrometer (TRIO 2, VG) in a shared, central facility on the UC Davis campus. Th instrument was old, and was not operational for extended periods of time. When it was in working conditi we were competing with other users for analytical time, and had very limited access to the instrument. For these reasons, we were only able to analyze 75 of the 660 samples during the two years in which we had originally expected to complete the work. Fortunately, we were able to raise funds to purchase our own ga chromatograph/mass spectrometer, (Shimadzu, QP 5000) which was installed in the Nutrition Department UC Davis in May 1996. Between May and November, 1996, we have completed the rest of the analyses from the previous study (~585 samples). Thus, we do not anticipate any problems in analyzing in a timely manner the number of samples that we are proposing to collect in the present study.

Timeline. The volunteer ward at the International Centre for Diarrhoeal Disease has the capacity to accommodate 20-24 subjects at a time. The estimated time required for a group of 20 subjects to complete the study protocol is 122 days, or approximately 4-5 months. During our previous studies of similar durati only 2 of 30 subjects dropped out prior to completion, both due to intercurrent infections. Recruitment an preliminary examinations of subjects will take place during the two months prior to each round. Thus, the clinical phase of the study will be carried out over a period of two years, with two groups of 20 subjects initiating the study protocol during the first year, and another group of 24 subjects during the second year. During each of the first two rounds (year 1), 20 subjects will be randomly assigned to one of four treatment groups (5/group). During the third round (year 2), 24 subjects will be randomly assigned to one of four treatment groups. Therefore, 16 subjects per treatment group will have completed the study protocol over the two year period. The plasma samples will be hand-carried to the University of California-Davis for measurement of the isotopic ratios of labeled/non-labeled retinol during years 1, 2 and 3, and the laboratory analyses will be ongoing. Data analysis and preparation of the final report will be completed by the end of the third year as outlined in Figure 4.

Limitations of the proposed study: Because of the sample size required and the limited capacity of the volunteer ward, this study will take nearly three years to complete. Moreover, because of budgetary constraints, we will only be able to evaluate the bioconversion of ß-carotene from one food item in addition to the comparison of ß-carotene and retinol. Because of this limitation, we have chosen to evaluate a green leafy vegetable because they are widely consumed and high in ß-carotene content. Recent studies have suggested that the bioconversion of ß-carotene from green leafy vegetables is particularly poor, (9, 34), however bioconversion has not been estimated quantitatively. Thus, although only one food will be evaluated over the study period, the vitamin A equivalency factor for spinach, which is representative of green leafy vegetables, in which carotenoids are found in protein-pigment complexes in chloroplasts, will b determined. We would like to evaluate the bioconversion of ß-carotene from a food source in which carotenoids are stored in chromoplasts, such as sweet potato. We are therefore seeking co-funding so that v can include a group that receives an equivalent amount of ß-carotene from sweet potato to compare the bioconversion of ß-carotene from plant sources in which carotenoids are stored in chromoplasts or chloroplasts.

The proposed abbreviated method for assessing relative bioavailability should permit future studies be conducted over a much shorter period of time. Thus, the information collected during the study period will be used to develop a more rapid method for evaluating the relative bioavailability of other carotenoid-rich foods, and the effects of dietary factors, cooking processes, and host factors on the bioconversion of provitamin A from selected foods in future studies.

The decision not to restrict volunteers to 24 hr observation in a closed unit might also be considered limitation of the study design. However, we found in our previous study that the subjects were very reliable in completing the protocol demands and their biochemical responses were fully consistent with the diets the received in the study ward. Thus, we do not think this aspect of the design will weaken the interpretation of the study. Use of the alternative design requiring subjects to remain in the ward for 24 hours would increas considerably the cost of the study and would seriously compromise our ability to recruit subjects.

A conceivable limitation relates to the possibility that bioconversion of carotenoids depends on the individual's vitamin A status. Although we will not be able to address this issue directly because of sample size limitations, we are restricting the study sample to individuals with presumably low vitamin A reserves, and we are attempting to balance the dietary groups by initial vitamin A status (based on baseline plasma retinol concentration). We will also control statistically for any baseline differences in retinol pool size, if necessary, when comparing the change in pool size by dietary group.

Finally, we do not believe that the abbreviated method that we are proposing for assessing bioconversion of ß-carotene to vitamin A is invalid, as suggested by a previous reviewer who was concerne that the doses of labeled vitamin A would affect the activity of the intestinal dioxygenase enzyme based on previous work done in rats (J Nutr 126:499, 1996). The activity of the intestinal cleavage enzyme was low in rats with high body stores of vitamin A than in rats with normal or low vitamin A body stores. The rats with high vitamin A body stores had been supplemented for 12 weeks with high doses (10x normal) of vitamin A before they were started on the experimental diets. Thus, their body stores of vitamin A were hi before they began receiving ß-carotene in the experimental diets. In our proposed study, subjects with low plasma retinol concentrations will be selected, and they will be kept on low-vitamin A diets for 21 days before they receive the dose of d8-retinyl acetate to determine whether the proposed abbreviated method ca be used to predict final vitamin A pool sizes. Thus, at that point in time, the subjects will have low body stores of vitamin A and they will be getting only a single dose (5 mg) of vitamin A before they start receiving their dietary treatments. Because their body stores of vitamin A are expected to be very low (~12 mg) initially, this small amount of vitamin A (5 mg) may increase their vitamin A stores, but their pool size will still be very low. Thus, we do not believe that a single, relatively small dose of vitamin A would be expected to have a significant effect on the activity of the intestinal cleavage enzyme.

Facilities Available

Describe the availability of physical facilities at the place where the study will be carried out. For clinical and laboratory-based studies, indicate the provision of hospital and other types of patient's care facilities and adequate laboratory support. Point out th laboratory facilities and major equipments that will be required for the study. For field studies, describe the field area including size, population, and means of communications. **(TYPE WITHIN THE PROVIDED SPACE).**

The laboratory analyses will be performed in the Department of Nutrition of the University of California-Davis which is equipped with several HPLCs with variable wavelength UV detectors (Hewlett Packard 1100, and Varian 5000) and a Shimadzu QP-5000 Gas Chromatograph/Mass Spectrometer. The laboratories are also equipped with fume hoods and accessories for extraction and sample preparation, uv/vis spectrophotometers, and -80 C freezers to support the proposed research.

The laboratory analyses in Bangladesh will be completed in the Biochemistry and Nutrition Laboratory of ICDDR,B. Aliquots of all specimens will be saved for validation of a random subset of samples in Davis. The following instruments will be available to the project: 1) a Waters HPLC system, which includes a model 510 HPLC pump, a model 712 WISP auto-injector, a model 481 spectrophotometer, and a model 745 data module; 2) a Pye-Unicam model SP8- 400 UV/VIS scanning spectrophotometer; and 3) assorted analytical balances, centrifuges, and spectrophotometers, a Waters millipore system for filtering solvents, and a Savant speed-vac (mode SC-110) for rapid evaporation of extracts.

Statistical analyses will be completed in Davis at the Data Center of the Program for International Nutrition. This Center contains 4 IBM compatible microcomputers, and all necessary data entry and analysis programs including PC-SAS (R) Release 6.04, dBase IV version 4.0 and SAAM II.

Data Analysis

Describe plans for data analysis. Indicate whether data will be analyzed by the investigators themselves or by other professionz Specify what statistical softwares packages will be used and if the study is blinded, when the code will be opened. For clini trials, indicate if interim data analysis will be required to monitor further progress of the study. **(TYPE WITHIN THE PROVID SPACE).**

Total body stores of vitamin A will be estimated before and after the supplementation perioc using the method of Furr et. al. (36) and plasma isotopic ratios 20 and 21 days after receipt of deuterated retinol (protocol days 26 and 27). The mean change in total body stores (post-supplementation - baseline) will be compared within treatment groups using the paired-t test, and across treatment groups using analysis of covariance with baseline vitamin A pool size as the covariate (non-parametric methods will be used if necessary). Regression analysis will also be used to predict estimated change in pool size with 5-10 day plasma isotopic ratios of retinol-d8/retinol during the supplementation period. This will enable us to determine whether plasma isotopic ratios during the supplementation period can predict the relative bioavailability of different dietary sources of vitamin A.

Ethical Assurance for Protection of Human Rights

Describe in the space provided the justifications for conducting this research in human subjects. If the study needs observations c sick individuals, provide sufficient reasons for using them. Indicate how subject's rights are protected and if there is any benefit risk to each subject of the study.

The present study involves non-ill adult male subjects who will be provided a balanced diet that meets full caloric requirement. Although the control subjects will be provided with a diet low in vitamin A they will also receive labeled vitamin A throughout the study period. These subjects are therefore unlikely to develop vitamin A deficiency during the study. If, however, any subject develops clinical symptoms of vitamin A deficiency, he will be treated with vitamin A. All subjects will be examined weekly by a physician to ensure their physical well being.

'Informed consent' will be obtained from the subjects after explaining in vernacular about the procedure they will undergo, the possible risks involved and also about their right to withdraw from the study at any time after the commencement. The study protocol has already been approved by the Institutional Review Board at the University of California-Davis and will be submitted to the Ethical Review Committee of ICDDR,B following its approval by the Research Review Committee.

Use of Animals

Describe in the space provided the type and species of animal that will be used in the study. Justify with reasons the use of particular animal species in the experiment and the compliance of the animal ethical guidelines for conducting the proposed procedures.

No animals will be used for this study.

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list Literature Cited. There is no page limit for this section, however exercise judgment in assessing the "standard" length.

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Dissemination and Use of Findings

Describe explicitly the plans for disseminating the accomplished results. Describe what type of publication anticipated: working papers, internal (institutional) publication, international publications, international conferences and agencies, workshops etc. Mention if the project is linked to the Government of Bangladesl through a training programme.

When sufficient data is available, the findings will be presented initially in one of the International Scientific Conferences on relevant issues of nutrition. In addition, the results may be used elsewhere including workshops, seminars, etc. as appropriate. Eventually the paper will be submitted for publication i a peer-reviewed journal on nutrition.

Collaborative Arrangements

Describe briefly if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement betwe the applicant or his/her organization and the collaborating organization. **(DO NOT EXCEED ONE PAGE)**

This project will be carried out as a collaborative effort of two administrative units: the Department of Nutrition and the Program in International Nutrition (PIN), University of California, Davis (KH Brown, Director of PIN and PI of proposal) and the Clinical Sciences Division of the International Centré for Diarrhoeal Disease Research, Bangladesh (G. Fuchs, Director). A letter of intent to collaborate has been provided by Dr. Fuchs and is included in Appendix 3.

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this pag for each investigator.

Name	Position	Date of Birth
Dr. Kazi Mohammad Asif Jamil	Senior Medical Officer, CSD ICDDR,B,; Dhaka, Bangladesh	1st January, 1964

Academic Qualifications (Begin with baccalaureate or other initial professional education)

Institution and Location	Degree	Year	Field of Study
Chittagong Medical College	M.B.B.S	1988	Medicine
University of Tokyo School of Medicine	PhD	1995	Medical Scienc (Nephrology)

Research and Professional Experience

Concluding with the present position, list, in chronological order, previous positions held, experience, and honours. Indicate current membershi on any professional societies or public committees. List, in, chronological order, the titles, all authors, and complete references to all publicatio during the past three years and to representative earlier publications pertinent to this application. **(DO NOT EXCEED TWO PAGES, USE CONTINUATION SHEETS).**

1989 - 1989: One year internship training in Chittagong Medical College Hospital, Chittagong, Bangladesh

1990 - 1991: Research fellow at the University of Tokyo School of Medicine, Tokyo, Japan

1991 - 1995: Attended PhD course at the Graduate School of University of Tokyo School of Medicine

1995 - 1997: Assistant Professor of Medicine and Physiology at University of Science and Technology Chittagong, Bangladesh

1997 - present : Senior Medical Officer, Clinical Sciences Division, ICDDR,B, Dhaka, Bangladesh

PROFESSIONAL MEMBERSHIP: Member of the International Society of Nephrology

Bibliogrphy

1. Expression of platelet activating factor receptor in renal tubular cell line (LLC-PK1). K.M.A.Jamil, T.Takano, A.Nakao, Z.Honda, T. Shimizu, T.Watanabe and K.Kurokawa. Biochem. Biophys. Res.Commu 187: 767-772, 1992.

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Name	Position	Date of Birth
Dr. Kenneth H. Brown	Porfessor, Department of Nutrition, University of California, Davis, USA	

Academic Qualifications (Begin with baccalaureate or other initial professional education)

Institution and Location	Degree	Year	Field of Study
Columbia University, New York, NY, USA	B.A.	1968	Pre-Med
University of Pennsylvania, Philadelphia, PA, USA	M.D.	1973	Medicine
Research and Professional Experience			

Concluding with the present position, list, in chronological order, previous positions held, experience, and honours. Indicate current membershi on any professional societies or public committees. List, in, chronological order, the titles, all authors, and complete references to all publicatio. during the past three years and to representative earlier publications pertinent to this application. **(DO NOT EXCEED TWO PAGES, USE CONTINUATION SHEETS).**

1986 – 1989: Associate Professor, Division of Human Nutrition, Department of International Health, Johns Hopkins University School of Health and Hygiene and Public Health, and Associate Professor, Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland

1989 – present : Professor, Department of Nutrition, University of California, Davis, California, USA, and Director, Program in International Nutrition.

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Name	Position	Date of Birth
Marjorie J. Haskell	Postdoctoral fellow, Department of Nutrition University of California, Davis, USA	

Academic Qualifications (Begin with baccalaureate or other initial professional education)

Institution and Location	Degree	Year	Field of Study
Western Kentucky University Bowling Green, KY, USA	B.A.	1980	French
University of Massachusetts, Amherst, MA USA	B.S.	1985	Nutrition
University of North Carolina, Chapel Hill, NC, USA	MPH	1987	Nutrition
University of California, Davis, CA, USA	Ph.D.	1996	Nutrition

Research and Professional Experience

Concluding with the present position, list, in chronological order, previous positions held, experience, and honours. Indicate current membership on any professional societies or public committees. List, in, chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. **(DO NOT EXCEED TWO PAGES, USE CONTINUATION SHEETS).**

February 1985 – August 1985: Laboratory Technician, Nutrition Evaluation Laboratory, USDA Human Nutrition Evaluation Laboratory, Tufts University, Boston, MA, USA

January 1986 – April 1986: Dietetic Intern, Duke University Medical Center, Durham, North Carolina, USA

June 1987 – June 1988: Research Assistant, Vitamin Bioavailability Laboratory, USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA, USA

February 1989 – September 1990: Research Assistant, Center for Studies of Sensory Impairment, Aging, and Metabolism, Guatemala City, Guatemala.

September 1991 – June 1992: Teaching Assistant, Department of Nutrition, University of California, Davis, CA, USA

September 1991 – August 1992: Research Assistant, Department of Nutrition, University of California, Davis, CA, USA

September 1992 – June 1996: Postgraduate researcher, Department of Nutrition, University of California, Davis, CA, USA

July 1995 – October 1995: Consultant, International Atomic Energy Agency, Vienna, Austria

July 1996 – present: Postdoctoral fellow, Department of Nutrition, University of California, Davis, CA, USA

Bibliogrphy

1. Haskell MJ, Handelman GH, Peerson JM, Jones AD, Rabbi A, Awal A, Wahed MA, Mahalanabis D, and Brown KH. Assessment of vitamin A status by the deuterated retinol dilution technique and comparison with hepatic retinol concentration in Bangladeshi surgical patients. Am J Clin Nutr 1997;66:67-74.

2. Haskell MJ, Islam A, Peerson JM, Handelman GJ, Wahed MA, Mahalanabis D, and Brown KH. Plasma kinetics of an oral dose of d4-retinyl acetate in healthy American and Bangladeshi subjects. Am J Clin Nutr, 1997 (in press).

3. Haskell MJ, Handelman GJ, Peerson JM, Ahmed A, Rabbi A, Awal MA, Wahed MA, Mahalanabis D, Burri BJ, Brown KH. Comparison of indirect vitamin A assessment techniques with hepatic vitamin A concentration in Bangladeshi surgical patients. Submitted, 1997.

4. Mason JB, Shoda R, Haskell M, Selhub J, Rosenberg IH. Carrier affinity as a mechanism for the pH-dependence of folate transport in the small intestine. Biochemica et Biophysica Acta, 1990;1024:331-335.

Abstracts

1. Brown KH, Haskell MJ, Mazumder RN, Jones AD, Peerson JM, Wahed MA, Mahalanabis D. Use of the deuterated retinol dilution technique to assess total body vitamin A reserves of adult volunteers consuming different levels of vitamin A. XVIII IVACG Meeting, Cairo, Egypt, 1997.

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5. Haskell M, Rivera C, Valdez C, Buluz J, Solomons NW. Riboflavin intake and nutritional status among rural and urban Guatemalan school children. The FASEB J, 4:1990.

Name	Position	Date of Birth
Dr. George J. Fuchs	Interim Director, International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh	

Academic Qualifications (Begin with baccalaureate or other initial professional education)

Institution and Location	Degree	Year	Field of Study
University of Missouri – Columbia Columbia, Missouri 65212, USA	B.A.	1974	Zoology
University of Missouri – Columbia Columbia, Missouri 65212, USA	M.D.	1980	Medicine

Research and Professional Experience

Concluding with the present position, list, in chronological order, previous positions held, experience, and honours. Indicate current membership on any professional societies or public committees. List, in, chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. **(DO NOT EXCEED TWO PAGES, USE CONTINUATION SHEETS).**

July – December 1980: Internship in Internal Medicine at Department of Internal Medicine, Good Samaritan Hospital, Phoenix, Arizona 85062

January – June 1881: Internship in Pediatrics at the department of Pediatrics, Phoenix Hospital Affiliated Pediatric Program, Phoenix, Arizona 85062

July 1981 – June 1983: Residency in Pediatrics in Tufts – New England Medical Center, Boston Floating Hospital for Infants and Children, Boston, Másachusetts 02111, USA

July 1983 – June 1984: Clinical and research fellow at Division of Geographic Medicine and Pediatric Gastroenterology/Nutrition, Tufts – New England Medical Center, Boston, Massachusetts 02111, USA

July 1984 – June 1986: Clinical and research fellow in Pediatric Infectious Diseases at University of Texas Health Science Center at Houston, Houston, Texas, USA

1986 – 1992: Assitant Professor of Pediatrics, Louisiana State University School of Medicine at New Orleans

1992 – 1997: Associate Professor of Pediatrics, Louisiana State University School of Medicine at New Orleans

1995 – 1997: Director, Clinical Sciences Division, International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

1998: Interim Director, International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

1998: Professor of Pediatrics, Louisiana State University School of Medicine at New Orleans

Bibliogrphy:

1. Fuchs GJ, Grand RJ, and Motil KJ: Malnutrition and Nutritional Support in Inflammatory Bowel Disease. *Nutritional Support Service* 5:28-33, 1985.

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* Principal Investigators: Jamil, Kazi Mohammad Asif & Brown, Kenneth H

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• Principal Investigators: Jamil, Kazi Mohammad Asif & Brown, Kenneth H

Detailed Budget for New Proposal

Project Title: Assessment of carotenoid bioavailability from plant sources

Name of PIs: Kenneth H Brown and Kazi Mohammad Asif Jamil

Protocol Number: Name of Division: Clinical Sciences Division

Funding Source: United States Department of Agriculture (available), and Micronutrient Initiative (expected)
Amount Funded (direct): US$ 146,000 Total: US$ 270,824 Overhead (%):US$ 54,165 (25%)

Starting Date: January 1999 Closing Date: December 2001

Strategic Plan Priority Code(s):

Sl. No	Account Description	Salary Support			US $ Amount Requested		
	Personnel	**Position**	**Effort%**	**Salary (Total)**	**1st Yr**	**2nd Yr**	**3rd Yr**
	Personnels at UC Davis:						
	Kenneth H Brown (UCD)	PI	20%, 20%,20%	0	0	0	0
	Marjorie J Haskell (UCD)	Co-investigator	50%, 50%, 10%	45,897	20,600	21,012	4,286
	Study Asst (UCD)		25%, 25%, 10%	17,725	7,276	7,421	3,028
	J M Peerson (UCD)	Statistician	5%, 5%, 5%	8,320	2,666	2,772	2,882
	Adm Asst (UCD)		5%, 5%, 5%	4,033	1,318	1,344	1,371
	Fringe benefits			14,815	6,212	6,347	2,256
	Subtotal			**90,791**	**38,072**	**38,896**	**13,823**
	Personnels at ICDDR,B:						
	Kazi Mohammad Asif Jamil	PI	50%, 50%, 20%	12,430	4,900	5,330	2,200
	George J Fuchs	Co-investigator	10%, 10%, 5%	0	0	0	0
	Lab supervisor		5%, 5%	1,800	900	900	
	Lab technician		100%, 50%	5,400	3,600	1,800	
	Dietitian		100%, 50%	4,500	3,000	1,500	
	Cooks		140%, 70%	3,969	2,646	1,323	
	Cleaner		100%, 50%	2,835	1,890	945	
	Sub Total			**30,934**	**16,936**	**11,798**	**2,200**

			1st Yr	2nd Yr	3rd Yr
	Consultants				
	Local Travel	Subject recruitment (ICDDR,B)	250	250	
	International Travel	3 Round trip (Sac-Dhaka)	4,800	2,400	
		Perdiem (90d @ $50/day)	2,000	1,500	
		Travel to scientific meeting			1,000
	Sub Total :		**7,050**	**4,150**	**1,000**

Supplies and Materials (Description of Items)

		1st Yr	2nd Yr	3rd Yr
	To be procured at UC Davis:			
	Vacutainers, needles, transfer pipets, storage vials, etc ($4/sample; 300 screening; 1120 Study samples	3,360	1,680	
	2 g d4RA @ $4,000/g	4,000	4,000	
	1 g d8RA @ $6,500/g	6,500		
	Solvent/lab supplies	1,250	1,250	
	Columns (2 HPLC @ $300, 1GC @ $250)	850		
	Reagents (standards, derivatizing reagents, NIST control serum, etc.)	600	600	
	GC/MS service contract	750	1,500	1,500
	Sub Totals	**17,310**	**9,030**	**1,500**
	To be procured at ICDDR,B:			
	Medicines (treatment of parasites)	188	188	
	Kitchen supplies	250		
	Food ($3/d x 9760 subject-days)	15,616	7,808	
	Subjective incentive ($3/d x 9760 subject-day)	15,616	7,808	
	Sub Totals	**31,670**	**15,804**	

	Other Contractual Services			
	Repair and Maintenance			
	Rent, Communications, Utilities ($60/m x 30 mos)	720	720	180
	Training Workshop, Seminars			
	Printing and Publication			500
	Staff Development			
	Sub Total	**720**	**720**	**680**

	Interdepartmental Services	**1st Yr**	**2nd Yr**	**3rd Yr**
	Computer Charges : ICDDR,B and UC Davis	350	350	100
	Pathological tests : at ICDDR,B (300 screening retinol, CRP, albumin, intestinal parasites)	2,250	2,250	
	Microbiological tests			
	Biochemistry Tests			
	X-Rays			
	Patients Study			
	Research Animals			
	Biochemistry and Nutrition			
	Transport			
	Xerox, Mimeographs etc.			
	Sub Totals	**2,600**	**2,600**	**100**
	Other Operating Costs			
	Capital Expenditure			

Total direct cost: 216,659 **Overhead cost (25%): 54,165**

Total project cost: 270,824

Note: USDA has recently granted US$ 146.000 for this project. The investigators have requested Micronutrient Initiative for further support and awaiting its reply.

Budget Justifications

Please provide one page statement justifying the budgeted amount for each major item. Justify use of man power, major equipment, and laboratory services.

Personnel: *University of California – Davis:* Dr. Marjorie Haskell will be responsible for final purification of plasma samples to isolate retinol and for the mass specrtometry analyses in Davis. In addition she will assist in setting up the project in Bangladesh, and will supervise laboratory analyses that will be conducted in Bangladesh. The doctoral student will assist with the laboratory analyses at in Davis. The statistician will complete the statistical analyses. The administrative assistant will be responsible for purchasing and shipping supplies, maintaining communication with Bangladesh, assisting with travel arrangements, and reviewing all financial interactions between the University of California and the field site. Additionally, the administrative assitant will type all correspondence and prepare all materials for publication.

ICDDR,B: Dr. Jamil will be responsible for conducting the whole study at ICDDR,B under the supervision of Professor George Fuchs. He will conduct physical examination of the study subjects and obtain all clinical specimens, as required. A lab technician will assist with all laboratory analyses and with preliminary processing of samples for subsequent mass spectrometry analysis. The dietitian will prepare the individual daily menus, supervise the preparation of foods, and determine the quantities of food items consumed by each subject at each of the meals. This person will also be responsible for preparation of duplicate diets to assess their vitamin A content. The cooks are budgeted at 140% of the salary for the first year because they will be working on weekends. The cleaners are self-explanatory.

Supplies: The blood drawing supplies are self-explanatory. A total of 1.28 g of d4-retinyl acetate and 0.8 g of d8-retinyl acetate will be required to complete the study, the remaining 0.72 g of d4-retinyl acetate and 0.45 g of d8-retinyl-acetate will be used for various standardization exercises, and to check for isotopic and chemical purity of the material. The solvents are those that are required for extraction and isolation of retinol from the plasma samples as explained in the proposal, and for the plasma carotenoids analyses. One column will be required for the GC/MS, and two columns for the HPLC in Davis. The other supply ietms should be self-explanatory.

Travel: Three round trip air fares from Sacramento to Dhaka have been budgeted, one for Dr. Brown and two for Dr. Haskell who will travel to Bangladesh to assist with setting up the first round of the project during the first year, and the final round in the second year.

APPENDIX 1

International Centre for Diarrhoeal Disease Research, Bangladesh
Voluntary Consent Form

Title of the Research Project: Assessment of carotenoid bioavailability from plant sources

Principal Investigators: Kenneth H Brown and Kazi Mohammad Asif Jamil

Before recruiting into the study, the study subject must be informed about the objectives, procedures, and potential benefits and risks involved in the study. Details of all procedures must be provided including their risks, utility, duration, frequencies, and severity. All questions of the subject must be answered to his/ her satisfaction, indicating that the participation is purely voluntary. For children, consents must be obtained from their parents or legal guardians. The subject must indicate his/ her acceptance of participation by signing or thumb printing on this form.

HSRC Log No. _____

Informed Consent to Participate in a Research Study

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
AND UNIVERSITY OF CALIFORNIA, DAVIS

Title of Study: Assessment of carotenoid bioavailability from plant sources.

Investigators Names, Depts, phone #'s: Dr. K M A Jamil, International Centre for Diarrheoal Disease Research, Bangladesh, tel. 871751-60 Ext 2314 and Kenneth H. Brown, M.D., Department of Nutrition, UC Davis, tel. (530) 752-1992

Purpose

You are being asked to participate in a research study. Investigators at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) in Dhaka and at the University of California, Davis (UC Davis) are conducting a study to learn more about the absorption of vitamin A from foods. Vitamin A is found in a wide variety of foods, however, very little is known about the absorption of vitamin A from vegetables and fruits. In this study, we hope to learn whether your body can absorb vitamin A from different food items. This information is needed to determine which foods are good sources of vitamin A.

Procedures

If you decide to volunteer, we will ask you to consume all of your meals during the next four months in the study cafeteria. You will also receive a vitamin and mineral supplement daily. These meals and the vitamin and mineral supplements that you will be given will satisfy all of your nutrient requirements except for vitamin A. After approximately one month of consuming your meals in the study cafeteria, you will begin receiving either a capsule containing 1.5 mg of vitamin A, or a serving of food containing the same amount of vitamin A, or a capsule containing no vitamin A every day for a period of 2 months. Whether you receive one of the capsules, or the serving of food will be determined by chance, as in a lottery. During the 2 month period, you will continue to consume all of your meals in the study cafeteria. After the 2 month period, you will continue to consume your meals in the study cafeteria for approximately one more month.

Additional procedures that will be completed are described as follows:

1. On the seventh day of the study, you will receive a small dose of "heavy vitamin A" by mouth, which will allow us to estimate how much vitamin A is present in your body. To complete this study, a small amount of blood (approximately 3 mL or about two teaspoons) will be drawn from a vein in your arm, on the morning that you receive the dose of "heavy vitamin A", and again 24 hours later, and again 20 and 21 days after you receive the dose.

2. On the 28th day of the study, you will receive another small dose of "heavy vitamin A" by mouth, which will allow us to estimate how much vitamin A you are absorbing from your food. A small amount of blood (approximately 8 mL or about two teaspoons) will be drawn from a vein in your arm 24 hours after you receive the dose, and again 5, 7, and 10 days following the dose.

3. On the 100th day of the study, you will receive the final oral dose of "heavy vitamin A", which will allow us to estimate the amount of vitamin A in your body again. This is necessary to determine how much vitamin A your body has absorbe A small amount of blood (~ 8 mL or about two teaspoons) will be drawn from a vein in your arm on the morning that you receive the dose, and again 24 hours later, and 20 and 21 days after you receive the dose.

Benefits

You may not benefit personally from participating in this study. However, your participation in the study will help us to understand how much vitamin A is absorbed from foods. This information will benefit society in general.

Risks

There are no major risks associated with your participation in this study. Risks of venipuncture include some discomfort bruising and rarely infection. The amount of blood that will be taken will not affect your health in any way. The special "heavy vitamin A" is a non-toxic substance which poses no risk to you. There is a slight risk that you may develop symptoms of vitamin A deficiency during the study. You will be examined by a physician weekly, and if any symptoms of vitamin A deficiency occur you will be treated with vitamin A. Upon completion of the study, volunteers who did not receive vitamin A during the study period will be given a single, high dose vitamin A supplement.

Right to Refuse or Withdraw

You may refuse to participate in the study without any consequences. You may change your mind about being in the study and quit after the study has started.

Questions

If you have any questions, please ask us. If you have additional questions later, either Dr. Fuchs, Dr. Brown or one of their assistants will answer them. Dr. Fuchs can be reached at the International Centre for Diarrhoeal Disease, Mohakhali, Dhaka Bangladesh, tel. 600171. Dr. Brown can be reached at 3150 Meyer Hall, UC Davis, Davis, CA 95616, tel. (916) 752-1992.

Participant's Initials _____

Confidentiality

The results from the tests will be analyzed by the investigators listed above, and will be available to these investigators only. Any information obtained in connection with the study will be used in a manner that does not publicly disclose your identity and will be kept confidential. Absolute confidentiality cannot be guaranteed, since research documents are not protected from subpoena.

Costs/Compensation

You will incur no financial costs as the result of your participation in this study. All meals will be provided to you free o charge. At the end of each 30-day period of participation in the study, you will receive $90 to compensate for the time devoted to the study. In the unlikely event that you are physically injured as a direct result of research procedures not done primarily for your own benefit, you will receive medical treatment at no cost. The International Centre for Diarrhoeal Disease Research, Bangladesh and the University of California do not provide any other form of compensation for injury.

YOUR SIGNATURE BELOW, WILL INDICATE THAT YOU HAVE DECIDED TO VOLUNTEER AS A RESEARCH SUBJECT AND THAT YOU HAVE READ AND UNDERSTOOD THE INFORMATION PROVIDED ABOVE AND THE BILL OF RIGHTS.

You will be given a signed and dated copy of this form to keep. You will also be given a copy of the Experimental Subject's Bill of Rights.

Date _____ _____
Signature of volunteer

Date _____ _____
Signature of investigator

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ
আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ ও ক্যালিফোর্নিয়া বিশ্ববিদ্যালয়, ডেভিস

সম্মতি পত্র

গবেষণার নাম ঃ শাকসব্জি থেকে মানবদেহে ক্যারোটিনয়েড আহরণ

গবেষকদের নাম ঃ

১। কাজী মোহাম্মদ আসিফ জামিল, আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ। ফোন ঃ ৮৭১৭৫১-৬০ /২৩১৪

২। কেনেথ এইচ, ব্রাউন, ক্যালিফোর্নিয়া বিশ্ববিদ্যালয়, ডেভিস, ইউ,এস,এ। ফোন নং (৫৩০) ৭৫২-১৯৯২।

উদ্দেশ্যঃ

আপনাকে একটি গবেষণায় অংশগ্রহণ করার জন্য আহবান করা হচ্ছে। আই,সি,ডি,ডি,আর,বি এবং ক্যালিফোর্নিয়া বিশ্ববিদ্যালয়ের গবেষকরা খাদ্য থেকে মানবদেহে ভিটামিন এ আহরণ সম্পর্কে বিশদ জানার জন্যে এই গবেষণা চালাবেন। বিভিন্ন ধরণের খাবারে ভিটামিন 'এ' পাওয়া যায়। কিন্তু সব্জি ও ফল থেকে কি পরিমান "ভিটামিন এ" আহরণ করা যায় সে সম্পর্কে আমাদের ধারণা অপ্রতুল। এই গবেষণার মাধ্যমে আমরা জানতে পারবো আপনার শরীর বিভিন্ন খাদ্য থেকে ভিটামিন 'এ' সংগ্রহ করতে পারে কিনা। কোন কোন খাদ্য ভিটামিন 'এ'-র উৎস হিসাবে উত্তম তা জানার জন্যে এই গবেষণার প্রয়োজন রয়েছে।

পদ্ধতি ঃ

আপনি যদি গবেষণায় স্বেচ্ছায় যোগ দিতে চান তাহলে আপনাকে আগামী চার মাস যে খাবার পরিবেশন করা হবে তার সবটুকু খেতে বলা হবে। আপনাকে প্রতিদিন সেই সঙ্গে ভিটামিন ও খনিজ সরবরাহ করা হবে যাতে ভিটামিন 'এ' ছাড়া অন্যান্য সকল পুষ্টিকর উপকরণ রয়েছে। একমাস ক্যাফেটিরিয়াতে খাওয়া দাওয়া করার পর আপনাকে দুইমাস ধরে প্রতিদিন ১.৫ মিঃগ্রাম ভিটামিন 'এ' অথবা সমপরিমাণ ভিটামিন 'এ' সমৃদ্ধ খাবার অথবা ভিটামিন 'এ' বিহীন ক্যাপসুল দেয়া হবে। আপনি কি ক্যাপসুলগুলির কোন একটি নাকি ভিটামিন সমৃদ্ধ খাবার পাবেন তা নির্ভর করবে লটারীর মত দৈবের উপর। এই দুইমাস ধরে আপনাকে ক্যাফেটেরিয়াতে দেয়া সবটুকু খাবার খেতে হবে। দুই মাস অতিক্রান্ত হবার পর আপনাকে আরো একমাস ক্যাফেটেরিয়াতে খেতে হবে। অন্যান্য প্রক্রিয়াগুলোর বিবরণ নীচে দেয়া হলোঃ-

১। গবেষণার ৭ম দিনে আপনাকে স্বল্প পরিমাণে ভারী ভিটামিন 'এ' খেতে দেয়া হবে, যার মাধ্যমে আপনার শরীরে কি পরিমাণ ভিটামিন 'এ' আছে তা নির্ণয় করা যাবে। এই পরীক্ষাটি সম্পন্ন করার জন্য আপনি যেদিন ভারী ভিটামিন 'এ' খাবেন ঐ দিন সকালে এবং তার ২৪ ঘন্টা পর এবং আবারো ২০ দিন ও ২১ দিন পর আপনার শিরা থেকে প্রতিবার ৮ মিঃ লিটার বা প্রায় দু-চামচ পরিমাণ রক্ত সংগ্রহ করা হবে।

২। গবেষণার ২৮তম দিনে আপনাকে 'ভারী ভিটামিন এ-র' আর এক ডোজ খাওয়ানো হবে যার মাধ্যমে আপনার শরীর খাবার থেকে কতটুকু ভিটামিন 'এ' আহরণ করেছে তা জানা যাবে। ভারী ভিটামিন 'এ' খাওয়ার ২৪ ঘন্টা অতিক্রান্ত হবার পর, এবং আবার ৫ দিন, ৭ দিন ও ১০ দিন পর আপনার শিরা থেকে অল্প পরিমাণে (৮ মিঃলি বা প্রায় দুই চা-চামচ পরিমাণ) রক্ত নেয়া হবে।

৩। গবেষণার ১০০তম দিনে আপনাকে 'ভারী ভিটামিন এ' শেষবারের মত খাওয়ানো হবে, যাতে করে আপনার শরীরে 'ভিটামিন এ'-র পরিমাণ আমরা আবারো নির্ণয় করতে পারি। আপনার শরীর কতটুকু ভিটামিন 'এ' গ্রহণ করতে পেরেছে তা জানার জন্যই এটার প্রয়োজন। আপনাকে যেদিন এটা দেয়া হবে ঐদিন সকালে, তার ২৪ ঘন্টা পর এবং আবারও ২০দিন ও ২১ দিন পর প্রতিবার ৮ মিঃলিটার বা প্রায় দু-চামচ পরিমাণ রক্ত আপনার শিরা থেকে নেয়া হবে।

উপকারিতা ঃ

এই গবেষণা থেকে আপনি হয়তো ব্যক্তিগতভাবে উপকৃত হবেন না। তবে আপনার এই গবেষণায় অংশগ্রহণের ফলে আমরা খাদ্য থেকে কি পরিমাণ ভিটামিন 'এ' মানবদেহে শোষিত হয় সে সম্পর্কে জানতে পারবো। এই তথ্য সমাজের সকলের উপকারে আসবে।

ঝুঁকি সমূহ ঃ

এই গবেষণায় অংশগ্রহণের ফলে আপনাকে কোন মারাত্মক ঝুঁকির সম্মুখীন হতে হবে না। শিরা থেকে রক্ত নেবার সময় সামান্য ব্যথা, ক্ষতসৃষ্টি বা কখনো কখনো জীবাণুর সংক্রমণ হবার ক্ষীন সম্ভাবনা থাকে। যেটুকু রক্ত আপনার শরীর থেকে নেয়া হবে তাতে আপনার স্বাস্থ্যের কোন ক্ষতি হবে না। বিশেষভাবে তৈরী ভারী ভিটামিন 'এ' শরীরের কোন ক্ষতি করে না।

গবেষণা চলাকালীন সময়ে আপনার দেহে ভিটামিন 'এ' র অভাব ঘটার ক্ষীন সম্ভাবনা আছে। আপনাকে প্রতি সপ্তাহে একজন চিকিৎসক নিয়মিত পরীক্ষা করবেন। যদি আপনার দেহে ভিটামিন 'এ' র অভাবজনতি চিহ্ন পরিলক্ষিত হয় তাহলে আপনাকে ভিটামিন 'এ' দিয়ে চিকিৎসা করা হবে। গবেষণা শেষ হবার পর, যারা প্রথম থেকে ভিটামিন 'এ' পাননি তাদেরকে একবার উচ্চমাত্রার ভিটামিন 'এ' খাওয়ানো হবে।

গবেষণায় অংশগ্রহণ প্রত্যাহার করার অধিকার ঃ

আপনি যে কোন সময় গবেষণা থেকে নাম প্রত্যাহার করতে পারেন। আপনি গবেষণায় অংশগ্রহণ করার ব্যাপারে মত পরিবর্তন করতে পারেন ও যে কোন সময় অব্যাহতি নিতে পারেন।

প্রশ্নোত্তর ঃ

আপনার কোন প্রশ্ন থাকলে অনুগ্রহ করে জিজ্ঞাসা করুন। আপনার মনে যদি পরেও কোন প্রশ্ন জাগে সেক্ষেত্রে আপনার প্রশ্নের উত্তর ডাঃ জামিল, ডাঃ ফুকস, ডাঃ ব্রাউন বা তাদের অধঃস্তন কোন ডাক্তার দিবেন। ডাঃ জামিলের ঠিকানা আইসিডিডিআর,বি, মহাখালী, ঢাকা। ফোন নং ৮৭১৭৫১-৬০/২৩১৪।

ডাঃ ব্রাউনের সঙ্গেও নিম্নের ঠিকানায় যোগাযোগ করা যাবেঃ 3150 Meyer Hall, UC Davis, Davis, CA 95618, Tel (916) 752-1992.

গবেষণায় অংশগ্রহণকারীর স্বাক্ষর

গোপনীয়তাঃ

পরীক্ষা নিরীক্ষার ফলাফল শুধুমাত্র উপরোক্ত গবেষকরাই জানতে পারবেন। এই গবেষণায় যা কিছু তথ্য পাওয়া যাবব তা এমনভাবে ব্যবহৃত হবে যাতে আপনার পরিচয় প্রকাশ না পায় এবং গোপনীয়তা রক্ষা হয়। পরিপূর্ণভাবে গোপনীয়তা রক্ষা করার নিশ্চয়তা অবশ্য দেয়া যায় না, কারণ গবেষণা কাজে প্রাপ্ত তথ্য আদালতের সাক্ষ্য প্রমাণের জন্য ব্যবহৃত হতে পারে।

মূল্য/ক্ষতিপূরণঃ

এই গবেষণায় অংশগ্রহণের জন্য আপনাকে কোন খরচ বহন করতে হবে না। আপনাকে বিনামূল্যে সমস্ত খাবার সরবরাহ করা হবে। গবেষণার ৩০দিন পার হলেই আপনাকে ৯০ ডলার ক্ষতিপূরণ দেয়া হবে। যদি কোন পর্যায়ে গবেষণা প্রক্রিয়ার মাধ্যমে আপনার শারীরিক কোন ক্ষতি হয়, যার সম্ভাবনা নেই বললেই চলে, তবে আপনাকে বিনামূল্যে চিকিৎসা করা হবে। আইসিডিডিআর,বি বা ক্যালিফোর্নিয়া বিশ্ববিদ্যালয় এছাড়া অন্য কোন ধরণের ক্ষতিপূরণ দিতে অপারগ।

আপনার নীচের স্বাক্ষরটি এই অর্থ বহন করবে যে আপনি স্বেচ্ছায় গবেষণায় অংশগ্রহণ করছেন এবং আপনি উপরে বর্ণিত তথ্যসমূহ এবং আপনার অধিকার সম্পর্কে পড়েছেন এবং বুঝতে পেরেছেন।

আপনাকে স্বাক্ষর ও তারিখ সহকারে এই দলিলের একটি কপি সংরক্ষণ করার জন্য দেয়া হবে। আপনাকে গবেষণা কাজে অংশগ্রহণকারী ব্যক্তির অধিকার সম্পর্কিত তথ্যও দেয়া হবে।

_____ _____

তারিখ অংশগ্রহণকারীর স্বাক্ষর

_____ _____

তারিখ গবেষকের স্বাক্ষর

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
Ma : ICDDR,B, GPO Box 128, Dhaka-1000, Ban csh
Phone : 871751-60, Telex : 675612 ICDD BJ
Fax : 880-2-883116, 886050, 871568, 871686, Cable : Cholera Dhaka

CENTRE
FOR HEALTH AND
POPULATION RESEARCH

November 10, 1996

Dr. Kenneth H Brown
Program in International Nutrition
Department of Nutrition, 3150 Meyer Hall
University of California
Davis

Dear Dr. Brown,

This is to inform you that ICDDR,B will be pleased to collaborate on the study entitled "Assessment of carotenoid bioavailability from plant sources, using paired tracer studies of total body retinol pool size; and validation of a simplified isotopic technique to estimate vitamin A equivalency of different dietary sources" and I will act as formal collaborator.

With best regards and looking forward to working with you.

Sincerely,

George Fuchs, MD
Director
Clinical Sciences Division

Appendix - IV

11 MAY 1998

Dear Colleague:

Again, I would like to congratulate you on your USDA/National Research Initiative Competitive Grants award. Enclosed is the panel summary and reviews of the proposal which you had submitted to the Improving Human Nutrition for Optimal Health Program. In addition, a relative ranking sheet is included so that you know in which priority category your proposal was placed. I hope that you find them helpful.

If you have any questions regarding the administration of your award, please do not hesitate to contact me.

Sincerely,

Kathleen C. Ellwood

Kathleen C. Ellwood, Ph.D.
Program Director
USDA/CSREES/NRI
STOP 2241
1400 Independence Ave., SW
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e-mail: kellwood@reeusda.gov

PANEL SUMMARY
IMPROVING HUMAN NUTRITION FOR OPTIMAL HEALTH

The panel decision regarding your proposal is based on the input provided by the reviews and the collected expertise and judgement of the individual panel members. This panel summary reflects the consensus opinion of the panel regarding your proposal.

Proposal #: 9800690
PI: Brown

Title: Assessment of Carotenoid Bioavailability from Plant Sources

POSITIVE ASPECTS OF PROPOSAL:

This is a novel and an interesting approach to answer an important question on the bioavailability of vitamin A from different sources and to determine vitamin A pool in humans. The PI has had a good amount of experience in the area of retinoids and carotenoids and is well capable of conducting this study in Bangladesh.

NEGATIVE ASPECTS OF PROPOSAL:

Since subjects in this study will be from a Bangladesh population who are marginally deficient in vitamin A, the panel had a concern on the applicability of information generated from this study to the U.S. population.

SYNTHESIS COMMENTS:

Panel believes that application of this isotope dilution method for identification of vitamin A pool is important and should be validated and tested in humans.

RELATIVE RANKING

PROPOSAL NO: 9800690

PROGRAM: IMPROVING HUMAN NUTRITION FOR OPTIMAL HEALTH

The panel ranked each proposal into one of six relative categories where "outstanding" represented the best proposals. This proposal was placed in the following category.

RECOMMENDED FOR FUNDING:		PERCENTAGE IN EACH CATEGORY:
☐	Outstanding	3
☐	High Priority	9
☑	Medium Priority	41
☐	Low Priority	25

NOT RECOMMENDED FOR FUNDING:		PERCENTAGE IN EACH CATEGORY:
☐	Having some merit but not recommended	10
☐	Not recommended	11

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Competitive Research Grants and Awards Management
National Research Initiative Competitive Grants Program
PROPOSAL REVIEW SHEET

Proposal No. 98-00690	Investigator Brown
Please return to: IMPROVING HUMAN NUTRITION FOR OPTIMAL HEALTH	

SEE GUIDELINES FOR REVIEW ON REVERSE

Comments: *(If needed, use additional sheets)* NOTE: Verbatim reviews, with reviewers' names removed but subject to editing by the Program Staff, will be sent to applicants.

Objectives: The investigators propose to use the deuterated retinol dilution (DRD) technique to quantitatively compare the bioavailability of β-carotene from spinach and from purified β-carotene in vitamin A-deficient men according to estimated increments in the total body pool of vitamin A. An ancillary objective is to develop an abbreviated DRD technique to enable rapid assessment of the relative bioavailability of β-carotene in various plant foods.

Scientific merit: The proposed investigation addresses a current controversy regarding the efficacy of interventions to reduce vitamin A deficiency through increased consumption of provitamin A carotenoids from vegetables.

Strengths:

Study population: Because study findings will be most directly applicable to vitamin A-depleted populations, use of subjects with low vitamin A reserves at the International Centre for Diarrhoeal Disease Research in Dhaka, Bangladesh is appropriate. However, a previous investigation reported a high incidence of coexisting conditions that affect vitamin A absorption and retention (Haskell et al. Am. J. Clin. Nutr. 1997;66:67-74).

Validation of the DRD technique: A strength of the application is the extensive validation of the DRD technique. The DRD technique is the only indirect method that provides a quantitative estimate of vitamin A stores. Thus the investigators will use the best available experimental approach to quantitatively compare increments in total body vitamin A resulting from daily ingestion of β-carotene from a purified supplement and from spinach. The investigators validated the DRD technique in a population with low to adequate body stores of vitamin A using surgical patients in Bangladesh (Haskell et al. Am. J. Clin. Nutr. 1997;66:67-74). A wide prediction interval was observed for estimates of hepatic vitamin A stores for individual subjects, but there was good agreement with hepatic vitamin A content measured by biopsy for the entire study population ($n = 30$). Estimated hepatic reserves were less similar for patients with hepatic vitamin A concentrations < 0.070 μmol/g, 0.077 ± 0.050 mmol (DRD) and 0.048 ± 0.017 mmol (biopsy) ($n = 13$). Although screened for malabsorption, three patients in this subgroup were later found to have clinical histories consistent with poor absorption and/or retention of the labeled retinol dose. In the current protocol, blood samples will be drawn 24 h after administration of the labeled dose so that poor absorption and/or retention of the dose will be detected and such subjects may be dropped from analysis. The investigators previously showed that the DRD technique is sufficiently sensitive to quantitate changes of total body vitamin A resulting from daily supplementation. In a study conducted in Bangladesh, highly significant differences in mean total body vitamin A were observed across three treatment groups after supplementation for 90 days with either 0, 1.5, or 3 mg RE of retinyl palmitate. This provides important experimental

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OSAL REVIEW SHEET

Proposal No. 98-00690	Investigator Brown
Please return to: IMPROVING HUMAN NUTRITION FOR OPTIMAL HEALTH	

SEE GUIDELINES FOR REVIEW ON REVERSE

ments: (If needed, use additional sheets) NOTE: Verbatim reviews, with reviewers' names removed but subject to editing by the Program Staff, will be sent to applicants.

support for the feasibility of the proposed study in which subjects will receive 1.5 mg RE daily for a 60-day period in the form of retinyl palmitate, purified β-carotene, or spinach.

Abbreviated DRD technique: The investigators propose a simplified procedure to estimate the relative bioavailability of provitamin A carotenoids from various plant foods. Subjects will receive an oral dose of 5 mg d8-retinyl acetate and the plasma retinol isotopic ratios will be measured 5-10 days after initiating supplementation with 1.5 mg RE in the form of retinyl palmitate, purified β-carotene, or spinach. In this revised protocol, the investigators will use a low dose (5 mg) of d8-retinyl acetate that is unlikely to have a significant impact on the efficiency of conversion of β-carotene to vitamin A over the succeeding 60-day supplementation period. The underlying assumption is that dilution of the d8-retinol in plasma will be proportional to the bioavailability of the ingested vitamin A source.

Weaknesses:

Plant source: Despite the complexity and duration of the project (3 years), information provided will be limited to the bioavailability of β-carotene from a single plant food, spinach, and from a purified supplement. The investigators will attempt to identify supplemental funding to expand the project to include an additional provitamin A-rich plant food.

Precision of the DRD technique: In the DRD validation study in Bangladesh, the investigators observed wide prediction intervals and concluded that the prediction model for hepatic vitamin A stores requires refinement for individual subjects. However, the DRD technique was shown to provide a good estimate of hepatic vitamin A reserves for a sample population. The technique is thus expected to be adequate to estimate mean increments in the body vitamin A pool across subjects.

Investigators and Institutional Capabilities: The investigators have demonstrated proficiency with regard to implementation of the DRD technique in the proposed study population. The clinical facilities at the International Centre for Diarrhoeal Disease Research and the analytical facilities at the University of California-Davis, including GC/MS capabilities, are well-suited to the proposed investigation.

Relevance: The project is directly relevant to the objective to enhance understanding of foods and food components in promoting health.

Other considerations: None

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OSAL REVIEW SHEET

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SEE GUIDELINES FOR REVIEW ON REVERSE

nments: (If needed, use additional sheets)

NOTE: Verbatim reviews, with reviewers' names removed but subject to editing by the Program Staff, will be sent to applicants.

Recommendation: The project is expected to provide important information regarding equivalency factors used to estimate the vitamin A contribution of provitamin A carotenoids from dark-green leafy vegetables in a vitamin-A deficient population. The only significant weakness is that bioavailability data will be provided for a single plant food.

REVIEWER: 2/pag

USDA, Cooperative State Research, Education, and Extension Service
Competitive Research Grants and Awards Management
National Research Initiative Competitive Grants Program
PROPOSAL REVIEW SHEET

Proposal No. 98-00690 | Investigator Brown

Please return to: IMPROVING HUMAN NUTRITION FOR OPTIMAL HEALTH

SEE GUIDELINES FOR REVIEW ON REVERSE

Comments: *(If needed, use additional sheets)*

NOTE: Verbatim reviews, with reviewers' names removed but subject to editing by the Program Staff, will be sent to applicants.

SCIENTIFIC MERIT: This application by Dr. Kenneth H. Brown proposes investigations in humans of the bioavailability of β-carotene from plant food sources. The studies will be carried out in Bangladesh using healthy male adults who have very low vitamin A stores. The overall goal of the proposal is to establish definitively in humans how much dietary β-carotene provided in green and leafy vegetables is converted to vitamin A. These data will be used to establish a valid conversion factor for β-carotene in plant food sources to vitamin A. Such information is needed to help resolve many inconsistencies in the literature regarding the conversion of provitamin A carotenoids to vitamin A.

It appears that most of the methodologies needed to carry out the proposed investigations are already in place. The PI has extensive expertise in the types of studies that he is proposing. The investigators will randomly assign healthy prescreened human volunteers to one of 4 groups. These groups consist of a group receiving a supplement of preformed vitamin A in corn oil; a second group receiving a supplement (providing the same amount of retinol equivalents as received by the preformed vitamin A supplement group) of purified β-carotene in corn oil; a group receiving the same amount of β-carotene in cooked spinach; and a control group provided only the vehicle corn oil. All groups will be provided a dose of deuterated retinyl acetate for use in assessing vitamin A status and changes in status. Vitamin A status will be assessed using Dr. Brown's deuterated-retinol dilution technique and HPLC purification of retinol followed by GC-MS detection. The experimental plan seems reasonable and the study should provide information that will be useful for understanding the bioavailability of β-carotene from plant food sources in free living human beings.

Although the study plan is strong and the question being asked is important, there are some potential problems with the study which are not thoroughly considered in the application. There are data in the literature which indicate that humans have markedly different capacities for β-carotene absorption and conversion. One wonders whether the 16 individuals per treatment group that the PI expects to recruit will provide the study with significant power if significant inter-individual differences are observed. Although the population undergoing study is presumably ethnically homogeneous, it is possible that genetic polymorphisms within a group might influence absorption and/or conversion of β-carotene. If significant differences are observed between individuals, how will this be dealt with and will this weaken the ability of the investigators to accomplish these stated goal.

Along the same line, there is a growing literature that some of the common dietary carotenoids can influence absorption and/or conversion of other carotenoids consumed in the same meal. Presumably, the investigators will have dietary records of the study participants. Will this be considered and is it possible that this will compromise the proposed studies?

PROPOSAL No. 98-00690—CONTINUED

Dr. Brown has published work carried out in Bangladesh previously and seems to have a successful track record for work carried out in Dhaka. However, there is very little consideration given in the application to how the PI and his colleagues in the US will supervise/quality control the work carried out in Bangladesh. Dr. George Fuchs of the ICDDR,Bangladesh provides a letter indicating that he will act as the "formal" collaborator to Dr. Brown. It appears from the CVs of Drs. Brown and Fuchs that they have not worked together previously (or at least published together). Although it seems likely, since the PI has carried out successful work in Bangladesh, that he will again be successful. However, the lack of consideration given to this point in the application is somewhat troubling. Since the success of the entire study hinges on the ability of the collaborators in Dhaka to carry out the protocols exactly as proposed in the application, the lack of consideration of safeguards, quality controls and management procedures for these studies suggests a somewhat too cavalier approach to a large, costly and important study. Along these lines, how will compliance with study guidelines be assessed, both for individual subjects and of the fidelity of the collaborators to adhere to the study design? Dr. Brown provides evidence from his previous studies in Bangladesh that compliance was not a problem for this work in Bangladesh; however, some consideration of this issue is needed by reviewers not familiar with working in Bangladesh.

Overall, this is an important study that will likely increase our understanding of carotenoid bioavailability from green and leafy vegetables for conversion to vitamin A in humans. This information is needed. The strengths of the study do substantially outweigh study weaknesses. The proposed investigations is worthy of USDA funding.

INVESTIGATORS AND INSTITUTIONAL CAPABILITIES: Dr. Brown and his colleagues in the US are experienced and well qualified for the sort of studies proposed in the application. The analytical protocols needed for the study seem to be in place in the US. Although the ICDDR, B is respected world wide, it is not clear from the application how work carried out in Bangladesh will be supervised and managed. The actual institutional capabilities available in Bangladesh available for these studies are not described. This raises some concerns in this reviewer regarding the ability of this team to complete the study. Although these concerns are not so large as to bring about the expectation that these studies will not be completed successfully, the concerns are real and a potential shortcoming of the study.

RELEVANCE: The proposed studies are directly relevant to the USDA. If completed successfully, they will help define the efficiency of absorption and conversion of β-carotene to vitamin A in humans.

OTHER CONSIDERATIONS: NONE.

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PROPOSAL REVIEW SHEET

Proposal No. 98-00690	Investigator Brown
Please return to: IMPROVING HUMAN NUTRITION FOR OPTIMAL HEALTH	

SEE GUIDELINES FOR REVIEW ON REVERSE

Comments: *(If needed, use additional sheets)* NOTE: Verbatim reviews, with reviewers' names removed but subject to editing by the Program Staff, will be sent to applicants.

Scientific Merit:

STRENGTHS: This work focuses upon comparison of the bioavailability of carotenes from spinach with that of purified beta carotene (BC) in human subjects using a deuterated retinol dilution (DRD) technique. The proposed studies are to be carried out in Dhaka, Bangladesh, an area where subjects are expected to have quite low vitamin A (VA) reserves. The results should shed important light on the BC conversion efficiences (assumed in humans to be 6:1) and the usefullness of spinach in providing VA value for undernourished populations. VA deficiency is a major world-wide problem although is less of a problem in the US. There is concern that BC from green leafy veretables may be quite poor. The research team appear to be well set up to carry out this work and have worked successfully together before. This team has already validated the DRD technique in Bangladesh in a similiar,low VA status population.

It was difficult to determine whether the "resonse to previous review" comments were appropriate without seeing the review. Never-the-less, the PI has attempted to appropriately detail the rational and study design.

WEAKNESSES: The data obtained will be relevant to a rather severely deficient population (12 mgs of total VA stores in this adult population would be considered as VA deficient). Information on this is important but it must be clear that VA sufficient populations will most likely yield vastly different conversion efficiencies of BC to VA and bioavailability figures.

The following concerns relate to the experimental design. There is a concern that if subjects only have about 12 mgs of total VA stores at the onset of the study, that provision of 10 mgs of VA acetate (twice during the study) will be a considerable pertibation of the total body VA pool kinetics. Also provision of a meal containing only 13.9 grams of fat may not optimize BC absorption, especially if 29 grams of fiber are also provided. This reviewer would urge that fat at a level of at least 20% of calories of the meal be provide. 29 grams of fiber in one meal is extremely high by US standards. Spinach also contains alpha carotene and B-cryptoxanthin, both precursors of VA although BC predominates. The limitation of capacity of subject numbers in Dhaka is of concern. Extending the study out to 36 months to complete the protocol is very expensive and may lead to uncontrolled changes in protocol. It would be cheaper and better controlled to carry out in the US and would be most relevant of NRI objects.

Investigators and Institutional Capabilities: This is a good team which can accomplish this work. However, the limitations of capacity of the volunteer ward is of concern, primarily from a cost standpoint.

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PROPOSAL REVIEW SHEET

Proposal No. 98-00690	Investigator Brown
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Comments: *(If needed, use additional sheets)* NOTE: Verbatim reviews, with reviewers' names removed but subject to editing by the Program Staff, will be sent to applicants.

Scientific Merit:

The PI intends to investigate vitamin A bioavailability from different dietary sources of β-carotene. The PI proposes using a novel technique (i.e., deuterated retinol dilution, DRD) to answer two important questions with the research described in this proposal:

- *Using the DRD method, can new more accurate conversion factors for estimating the bioconversion of provitamin A carotenoids to vitamin A in humans be established?;*
- *Could a simplier and faster approach based on relative plasma retinol isotopic ratio measured 5-10 after initiating a vitamin A or carotenoid-containing diet serve as a surrogate for the more elaborate and expensive DRD method?*

This proposal is a resubmission and it appears that the PI has addressed many of the previous reviewers' concerns. **This proposal has a number of strengths.** First, this proposal is very well written. The experimental objectives are clear. The reasons behind the design of each experiment is thoroughly justified. The basis for the justification for various elements of the experimental design is most often the preliminary data from the PI's lab. Thus the second major strength of this proposal is that the PI has conducted extensive preliminary studies with the DRD technique and has demonstrated that it is comparable to the more invasive liver biopsy approach (i.e., the gold standard method). Further, the PI has demonstrated that the DRD works for both depleted human subjects (i.e., the population that is to be studied in this proposal) and subjects with normal vitamin A stores. Third, the PI is uniquely qualified to conduct these studies. In particular, the PI has both the experience in the International Nutrition Research community and the collaborations that are essential for this research project to succeed.

In summary, this proposal is well-written and describes a novel experimental approach to address a long-standing and important nutrition question.

PI and Facilities:

The PI is a well-trained scientist and a recognized leader in the international nutrition research community. Over the last five years the PI has been highly productive, publishing over 50 peer-reviewed original research articles, many research committee reports and reviews on topics related to international nutrition.

The facilities available to the PI are suitable for carrying out the research as outlined. The recent purchase of a GC/MS instrument dedicated to the PI's research program will assure timely sample analyses.

Response to previous review

Response to panel summary:

1. The vitamin A supplements will be given with the noontime and evening meals. Those meals will be standardized such that the energy, protein, fat and fiber contents are equal across treatment groups. Subjects will be allowed to consume low-vitamin A and low-fat foods ad libitum at the morning meal and mid-afternoon snack to adjust for differences in individual caloric requirements. In our previous study, the body weights of the subjects at the end of the 3-month experimental period were not different from the baseline values, indicating that the subjects were able to self-adjust their caloric intakes to maintain their body weights.

2. The spinach will be steamed and then pureed. Soybean oil will be added to the puree to result in a fat content of 13.6 g/serving of spinach. Pureed spinach is commonly consumed in Bangladesh, and we do not anticipate any problems with acceptability.

3. The age range of the subjects has been reduced to 18-35 years.

Response to specific comments:

4. We do not believe that the abbreviated method that we are proposing for assessing bioconversion of ß-carotene to vitamin A is invalid. In the study that the reviewer cited (J Nutr 126:499, 1996), the activity of the intestinal cleavage enzyme was lower in rats with high body stores of vitamin A than in rats with normal or low vitamin A body stores. The rats with high vitamin A body stores had been supplemented for 12 weeks with high doses (10x normal) of vitamin A before they were started on the experimental diets. Thus, their body stores of vitamin A were high before they began receiving ß-carotene. In our proposed study, subjects with low plasma retinol concentrations will be selected, and they will be kept on low-vitamin A diets for 21 days before they receive the dose of d8-retinyl acetate to determine whether the abbreviated method can be used to predict final vitamin A pool sizes. Thus, at that point in time, the subjects will have low body stores of vitamin A and they will be getting a single dose (5 mg) of vitamin A before they start receiving their dietary treatments. Because their body stores of vitamin A are expected to be very low (~12 mg) initially, this small amount of vitamin A would not be expected to change the vitamin A stores to an extent that would effect the activity of the intestinal cleavage enzyme.

5. Ramadan will occur in Dec-Jan in 1998-9. We will schedule the clinical phase of the study so that there is no conflict with Ramadan.

6. We do not believe that non-compliance with the study protocol is a serious concern because in our previous study the estimated vitamin A pool size of the control group did not change significantly over time. The mean pool size for the control group was estimated as 11.4 ± 6.7 mg at baseline, and 13.8 ± 8.8 mg after consuming the low-vitamin A diet for a period of 90 days and receiving 15 mg of labeled vitamin A on days 1 and 90. The estimated pool sizes of the vitamin A supplemented groups are also consistent with good compliance to the study protocol, as described in the text.

7. In our previous study, subjects in the control group did not show any clinical signs of vitamin A deficiency after the 3-month experimental period. The mean estimated vitamin A pool size did not change after 90 days of consuming the low-vitamin A diet when compared with baseline values. The subjects will be examined by a physician weekly. If any subject shows clinical signs of deficiency he will be treated immediately. At the end of the 3-month period, subjects in the control group will receive 60 mg of retinyl palmitate in corn oil.

8. It is feasible to recruit 64 subjects for the study. For our previous study, we found 58/168 (35%) subjects with low plasma retinol values during our initial screening phase. We will screen ~300 individuals to identify 64 subjects with low plasma retinol values who are willing to participate in the study.

Check List

After completing the protocol, please check that the following selected items have been included.

1. Face Sheet Included X
2. Approval of the Division Director on Face Sheet [X]
3. Certification and Signature of PI on Face Sheet, #9 and #10 [X]
4. Table on Contents [X]
5. Project Summary [X]
6. Literature Cited [X]
7. Biography of Investigators [X]
8. Ethical Assurance [X]
9. Consent Forms [X]
10. Detailed Budget [X]