International Centre for Diarrhoeal Disease Research, Bangladesh
CENTRE FOR HEALTH AND POPULATION RESEARCH
Mail : ICDDR, B, GPO Box 128, Dhaka-1000, Bangladesh
Phone: 880-2-8811751-60, Telex : 642486 ICDD BJ
Fax : 880-2-8823116, 8812530, 8811568, 8826050, 9885657, 8811686, 8812529
Cable : Cholera Dhaka

Memorandum

CSD 2002

02 December 2002

To : Dr. KMA Jamil
Principal Investigator of protocol # 2002-032
Clinical Sciences Division

From: Professor Mahmudur Rahman
Chairman,
Ethical Review Committee (ERC)

Sub : Approval of protocol # 2002-032

Thank you for your memo dated 28 November 2002 with the modified version of your protocol # 2002-032 entitled "Estimation of the average Vitamin A requirement of adult males". The modified version of the protocol is hereby approved upon your satisfactory addressing of the issues raised by the ERC in its meeting held on 13 November 2002.

You shall conduct the study in accordance with the ERC-approved protocol; and shall be responsible for protecting the rights and welfare of the subjects and compliance with the applicable provisions of ERC Guidelines. You shall also submit report(s) as required under ERC Guidelines. Relevant excerpt of ERC Guidelines and 'Annual/Completion Report for Research Protocol involving Human Subjects' are attached for your information and guidance.

I wish you all success in running the above-mentioned study.

Thank you.

Copy: Acting Associate Director
Clinical Sciences Division

October 2002 ERC meeting minutes

International Centre for Diarrhoeal Disease Research, Bangladesh
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Cable : Cholera Dhaka

Memorandum

17 November 2002

To : Dr. KMA Jamil
Principal Investigator of protocol # 2002-032
Clinical Sciences Division

From : Professor Mahmudur Rahman
Chairman, Ethical Review Committee (ERC)

Sub : **Protocol # 2002-032**

Thank you for your protocol # 2002-032 entitled "Estimation of the average Vitamin A requirement of adult males" which the ERC considered in its meeting held on 13th November 2002. After review and discussion, the following observations were made on the protocol:

a) On the ERC Face Sheet, item # 2(a) should be marked 'YES'.

b) It was felt that 2-3 ml of blood should be enough for determination of the plasma vitamin A concentration and CRP.

c) The Bangla consent forms do not clearly indicate that there is no personal and direct benefits to the subjects in participating in the study.

d) In the English consent form, the words ' reasonably necessary medical treatment' should be replaced by the words 'necessary treatment free of cost'.

You are, therefore, advised to modify the protocol incorporating the above observations and submit the modified version of the protocol for consideration of the Chair.

Thank you.

Copy: Acting Associate Director
Clinical Sciences Division

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Cable : Cholera Dhaka

Memorandum

3 November 2002

To : Dr. K M A Jamil
Principal Investigator of protocol # 2002-032
Clinical Sciences Division

From: Professor Lars Åke Persson
Acting Chairman
Research Review Committee (RRC)

Sub : Approval of protocol # 2002-032

Thank you for your memo dated 3rd November 2002 with the modified version of your protocol # 2002-032 entitled "Estimation of the average Vitamin A requirement of adult males". The modified version of your protocol is hereby approved upon your satisfactory addressing of the issues raised by the RRC considered in its meeting held on 10th October 2002.

Thank you.

Copy: Acting Associate Director
Clinical Sciences Division

Attachment 1 Date: November 3, 2002

(FACE SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigators: 1. Kazi Mohammad Asif Jamil
2. Marjorie Haskell

Trainee Investigator (if any): _____

Application No. 2002-032

Supporting Agency (if Non-ICDDR,B): United States Department of Agriculture

Title of Study: Estimation of the Average Vitamin A Requirement of Adult Males

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 organs or 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
   X Protocol (Required)
   X Abstract Summary (Required)
   X Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw) (Required)
   X Informed consent form for subjects
   ____ Informed consent form for parent or guardian
   ____ Procedure for maintaining confidentiality
   ____ Questionnaire or interview schedule*

   * If the final instrument is not completed prior to review, the following information should be included in the abstract summary
   1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy
   2. 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.

Ajamil

Principal Investigator

Trainee

ABSTRACT SUMMARY

Rationale and purpose of the study

Current estimates of vitamin A requirements are based on very limited data obtained from two old studies conducted on a small number of subjects. The data from these two studies are very limited and highly variable. Stable isotope techniques are now available for safely measuring body stores of vitamin A in humans. We are proposing to use the paired deuterated retinol dilution (DRD) technique to estimate the amount of daily vitamin A intake that is required to maintain a constant total body vitamin A pool size. The level of daily intake at which vitamin A pool size is maintained will be used as an estimate of the average daily vitamin A requirement.

Methods

The study will be conducted in two rounds (or phases) and 75 subjects will be screened for each round. Healthy, Bangladeshi men (18-35 years of age) with low plasma retinol concentration (<35 μg/dL) will be selected for the study. In each round, 32 subjects will be selected on the basis of lowest levels of plasma retinol concentration. The subjects will be divided into two groups - Group 1 subjects will be maintained on a low vitamin A diet and supplemented with vitamin A in the dosage range of 100 – 1000 μg/day with the aim of determining the dose-response relationship in a population with a low vitamin A body store. These subjects will be randomly assigned to 1 of 8 treatment groups (n = 4/group) to receive either 100, 200, 300, 400, 500, 600, 800 or 1000 μg RE/day. The difference in vitamin A pool size before and after supplementation will enable us to calculate the increments in pool size in response to various doses of vitamin A.

Group 2 subjects will be recruited in the second round (phase) of the study using a similar screening procedure. But they will be repleted with four oral doses of 60 mg vitamin A at five-day intervals to achieve a level of vitamin A pool size that is comparable with North American adult males. These subjects will then be randomly assigned to 1 of 8 treatment groups (n = 4/group) to receive either 200, 500, 800, 1100, 1400, 1700, 2000, or 2300 μg RE/day. At the end of the study, the change in vitamin A pool size after supplementation will be assessed using DRD technique and the dose-response curve will be obtained.

The total vitamin A body store (or 'pool size') will thus be measured twice in each subject – before and after the 60-day supplementation with various doses of vitamin A as mentioned above. To estimate vitamin A pool size, the subjects will receive an oral dose of vitamin A labeled with non-radioactive stable isotope, [$^2H_4$]-retinyl acetate. 10 ml of blood will be drawn before administering the labeled vitamin A and then 1, 3, 20 and 21 days after giving the dose to measure plasma isotopic ratios of labeled to non-labeled vitamin A. In addition, 10 ml of blood will be drawn just after the end of supplementation period to measure plasma retinol concentration. Thus, blood will be

collected from each subject for 10 times (day 1, 3, 20, 21, 83, 94, 95, 97, 114, and 115) during the study period as shown in Fig 3 (Appendix-3).

All subjects will consume a basal diet with low vitamin A contents (75 – 100 μg/day) during the study period. However, they will be receiving various doses of vitamin A during the 60-day supplementation period as mentioned above. Group 1 subjects, who will be supplemented with vitamin A to maintain a low vitamin A body store (mean of ~30 mg) will receive a single high dose supplement (60 mg) of vitamin A at the end of the study.

Risks and benefits

The risks of venipuncture include some discomfort, bruising and rarely infection. The amount of blood that will be taken will not affect the health of the subjects under investigation in any way. Some subjects will be receiving vitamin A as low as 175-200 μg/d from the diet and capsules combined. However, it is very unlikely that clinical signs of vitamin A deficiency will occur at this level of intake. It may be mentioned here that in previous studies in which Bangladeshi men received 0 mg vitamin A/day for a period of 120 days, clinical symptoms of vitamin A deficiency did not occur in any of the subjects. The study physician will examine the subjects regularly to watch for any symptoms of vitamin A deficiency. Any subject who manifest clinical signs of vitamin A deficiency will be treated with vitamin A immediately. Some subjects may receive vitamin A supplements as high as 2300 μg/d. This amount is, however, below the Upper Limit (3000 μg/d) established by the Institute of Medicine.

The subjects may not benefit personally from participating in this study but the results of this study will benefit society in general because, at present, there is very little information on the amount of vitamin A that is needed to meet the body's needs.

Costs/Compensation

The subjects will incur no financial costs as the result of their participation in this study. They will receive monetary compensation for the time they will spend at the study facility. In the unlikely event that any child is physically injured as a direct result of research procedures, he/she will receive reasonable medical treatment at no cost.

Confidentiality

The results from the tests will be analyzed by the investigators listed above, and will be available to the investigators only. Any information obtained in connection with the study will be used in a manner that does not publicly disclose the identity of the subjects and will be kept confidential unless required by the court of law.

ICDDR,B: Centre for Health & Population Research **RRC APPLICATION FORM**

RESEARCH PROTOCOL

Protocol No. 2002-032

FOR OFFICE USE ONLY

RRC Approval: ☐Yes / ☐No Date:

ERC Approval: ☐Yes / ☐No Date:

AEEC Approval: ☐Yes / ☐No Date:

Project Title: Estimation of the Average Vitamin A Requirement of Adult Males

Theme: *(Check all that apply)*

- X Nutrition
- ☐ Emerging and Re-emerging Infectious Diseases
- ☐ Population Dynamics
- ☐ Reproductive Health
- ☐ Vaccine evaluation
- ☐ HIV/AIDS
- ☐ Environmental Health
- ☐ Health Services
- ☐ Child Health
- ☐ Clinical Case Management
- ☐ Social and Behavioural Sciences

Key words: vitamin A requirement, stable isotopes, deuterated retinol dilution (DRD)

Relevance of the protocol: The current estimates of vitamin A requirements are based on very limited data obtained from two old studies conducted on a small number of subjects. The data from these two studies are very limited and highly variable. Given that stable isotope techniques are now available for studying vitamin A metabolism safely in humans, we are proposing to use the paired deuterated retinol dilution (DRD) technique to estimate the amount of daily vitamin A intake that is required to maintain a constant total body vitamin A pool size. The level of daily intake at which vitamin A pool size is maintained will be used as an estimate of the average daily vitamin A requirement.

Principal Investigator 1 : Dr. K.M.A. Jamil **Division:** CSD **Phone:** 8811751-60/2333

Address: ICDDR,B, Mohakhali, Dhaka-1212, Bangladesh **Email:** jamil@icddrb.org

Principal Investigator 2 : Marjorie Haskell **Phone:** 1-530-752-1992

Address: Dept of Nutrition, University of California Davis, USA **Email:** mjhaskell@ucdavis.edu

Co-Investigator(s): Kenneth H. Brown, MD, Dept of Nutrition, University of California Davis, USA
M. A. Wahed, LSD, ICDDR,B

Collaborating Institute(s): University of California Davis, USA

Population: Inclusion of special groups *(Check all that apply)*:

Gender
- X Male
- ☐ Females

Age
- ☐ 0 – 4 years
- ☐ 5 – 9 years
- ☐ 10 – 19 years
- X 20 +
- ☐ > 65

- ☐ Pregnant Women
- ☐ Fetuses
- ☐ Prisoners
- ☐ Destitutes
- ☐ Service providers
- ☐ Cognitively Impaired
- ☐ CSW
- ☐ Others (specify)
- ☐ Animal

Project / study Site (*Check all that apply*:

- [X] Dhaka Hospital
- [ ] Matlab Hospital
- [ ] Matlab HDSS area
- [ ] Matlab non-HDSS area
- [ ] Mirzapur
- [ ] Dhaka Community
- [ ] Chakaria
- [ ] Abhoynagar
- [ ] Mirsarai
- [ ] Patyia
- [ ] Other areas in Bangladesh
- [ ] Outside Bangladesh
  name of country:
- [ ] Multi centre trial
  (Name other countries involved)

Type of Study *(Check all that apply)*:

- [ ] Case Control study
- [ ] Community based trial / intervention
- [ ] Program Project (Umbrella)
- [ ] Secondary Data Analysis
- [ ] Clinical Trial (Hospital/Clinic)
- [ ] Family follow-up study
- [ ] Cross sectional survey
- [ ] Longitudinal Study (cohort or follow-up)
- [ ] Record Review
- [ ] Prophylactic trial
- [ ] Surveillance / monitoring
- [X] Others

Targeted Population *(Check all that apply)*:

- [X] No ethnic selection (Bangladeshi)
- [ ] Bangalee
- [ ] Tribal groups
- [ ] Expatriates
- [ ] Immigrants
- [ ] Refugee

Consent Process *(Check all that apply)*:

- [X] Written
- [ ] Oral
- [ ] None
- [ ] Bengali language
- [ ] English language

Total sample size: 64

Determination of Risk: Does the Research Involve *(Check all that apply)*:

- [ ] Human exposure to radioactive agents?
- [ ] Fetal tissue or abortus?
- [ ] Investigational new device?
  (specify_____)
- [ ] Existing data available from Co-investigator
- [ ] Human exposure to infectious agents?
- [ ] Investigational new drug
- [ ] Existing data available via public archives/source
- [X] Pathological or diagnostic clinical specimen only
- [ ] Observation of public behaviour
- [ ] New treatment regime

Yes/No

- [X] [ ] Is the information recorded in such a manner that subjects can be identified from information provided directly or through identifiers linked to the subjects?
- [ ] [X] Does the research deal with sensitive aspects of the subject's behaviour; sexual behaviour, alcohol use or illegal conduct such as drug use?

Could the information recorded about the individual if it became known outside of the research:

- [ ] [X] a. place the subject at risk of criminal or civil liability?
- [ ] [X] b. damage the subject's financial standing, reputation or employability; social rejection, lead to stigma, divorce etc.

Do you consider this research *(Check one)*:

- [ ] greater than minimal risk
- [ ] no risk
- [X] no more than minimal risk
- [ ] only part of the diagnostic test

Minimal Risk is "a risk where the probability and magnitude of harm or discomfort anticipated in the proposed research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or tests. For example, the risk of drawing a small amount of blood from a healthy individual for research purposes is no greater than the risk of doing so as a part of routine physical examination".

Yes/No

X ☐ Is the proposal funded?

If yes, sponsor Name: United States Department of Agriculture (USDA)

Yes/No

☐ X Is the proposal being submitted for funding ?

If yes, name of funding agency: (1)

(2)

Do any of the participating investigators and/or their immediate families have an equity relationship (e.g. stockholder) with the sponsor of the project or manufacturer and/or owner of the test product or device to be studied or serve as a consultant to any of the above?

IF YES, submit a written statement of disclosure to the Director.

Dates of Proposed Period of Support	**Cost Required for the Budget Period (S)**		
(Day, Month, Year - DD/MM/YY)	***a. Ist Year***	***2nd Year***	***3rd Year***
Beginning date: January 2003	**65,152**	**59,978**	**2,939**
End date: March 2005	***b. Direct Cost :*** **107,621**	***Total Cost :*** **128,069**	

Approval of the Project by the Associate 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.

Dr M. A. Salam — Name of the Acting Associate Director

Signature

31/10/2002 — Date of Approval

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.

Signature of PI

Date: October 31, 2002

Name of Contact Person (if applicable): NA

Table of Contents

X 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 : 1. K.M.A. Jamil, ICDDR,B
2. Marjorie Haskell, UC Davis, USA

Project Name : Estimation of the Average Vitamin A Requirement of Adult Males

Total Budget: US$128,069 Beginning Date: Jan 2003 Ending Date: March 2005

It is very important to correctly estimate the daily vitamin A requirement in humans in order to make specific recommendations on daily intake of this micronutrient in different age groups. The current estimates of vitamin A requirements are based on very limited data obtained from two old studies conducted on a small number of subjects. The data from these two studies are very limited and highly variable. Given that stable isotope techniques are now available for studying vitamin A metabolism safely in humans, we are proposing to use the paired deuterated retinol dilution (DRD) technique to estimate the amount of daily vitamin A intake that is required to maintain a constant total body vitamin A pool size. The paired-DRD technique will be used to assess change in vitamin A pool size in response to supplementation with increasing levels of daily vitamin A intake. Mean change in vitamin A pool size (post-supplementation pool size minus initial pool size) will be calculated for each level of dietary vitamin A intake. To estimate the average vitamin A requirement, regression analysis will be used to fit a line to the data on change in pool size vs. level of daily vitamin A intake. The point at which the regression line crosses the x-axis will be used as an estimate of the level of vitamin A intake at which vitamin A pool size is maintained.

The study will estimate the amount of vitamin A required daily to maintain vitamin A pool size in two groups of subjects – the first group will be supplemented with different levels of vitamin A (100-1000 µg/d) with the aim of maintaining a low initial vitamin A body store (mean of ~30 mg). The second group will be supplemented initially with high doses of vitamin A to achieve a high vitamin A body store. These subjects will then be supplemented with vitamin A (200-2300 µg/d) with the aim of maintaining the same vitamin A status. Thus, if, at some point in the future, an "optimal" vitamin A pool size is identified based on specific functional outcomes, the results of this study will be useful in estimating the amount of daily vitamin A intake that would be required to maintain that pool size.

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

Name	Professional Discipline	Role in the Project
1. Kazi Mohammad Asif Jamil	Physician-scientist (Nutrition)	PI
2. Marjorie Haskell	Nutritionist	PI
3. Kenneth H Brown	Professor of Nutrition	Co-investigator
4. M. A. Wahed	Biochemist	Co-investigator

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. We will observe a linear response in mean change in vitamin A pool size following daily supplementation with different levels of vitamin A (100-1000 μg/d) in subjects with low initial vitamin A body stores (mean of ~30 mg).

2. We will observe a linear response in mean change in vitamin A pool size in response to daily supplementation with increasing levels of vitamin A (200-2300 μg/d) in subjects with larger initial vitamin A body stores (mean of ~130 mg). The estimated amount of daily vitamin A required to maintain vitamin A pool size in subjects with larger body stores will be greater than that required to maintain smaller body stores (mean of ~30 mg) because of higher vitamin A disposal rates in subjects with larger vitamin A body stores.

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 level of daily vitamin A intake that will maintain constant vitamin A body stores using the paired deuterated retinol dilution technique in Bangladeshi men with low initial total body stores of vitamin A (mean of ~30 mg).

2. To estimate the level of daily vitamin A intake that will maintain constant vitamin A body stores using the paired deuterated retinol dilution technique in Bangladeshi men with high initial total body stores of vitamin A (mean of ~130 mg).

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).**

Current estimates of vitamin A requirements are based on very limited data from two studies that were conducted more than 25 years ago in a small number of men who were depleted and then repleted with vitamin A (1, 2). In both studies, the estimated vitamin A requirements were based on the curative effect of small daily doses of vitamin A on symptoms of vitamin A deficiency. Specifically, the requirements were estimated as the amounts of daily vitamin A intake that are sufficient to 1) correct impaired dark adaptation, abnormal electroretinograms, and follicular hyperkeratosis among vitamin A-depleted subjects, and 2) increase plasma retinol concentrations to the normal range in vitamin A-depleted subjects (1, 2). Additionally, in one of the two studies, the subjects received a tracer dose of radiolabeled vitamin A, which allowed the investigators to estimate the daily vitamin A utilization rate. Thus, in that study, the requirements were based on an estimate of the amount of vitamin A that is utilized daily, in addition to the amount of vitamin A that is sufficient to reverse clinical symptoms of vitamin A deficiency. However, as described below, the data from these two studies are very limited and highly variable. For these reasons, the most recent version of the Dietary Reference Intakes (3) lists the collection of new information on which to base vitamin A requirements as a research priority. Given that stable isotope techniques are now available for studying vitamin A metabolism safely in humans, we are proposing to use the paired deuterated retinol dilution (DRD) technique to estimate the average daily vitamin A requirement of adult males. The DRD technique provides a quantitative estimate of total body vitamin A stores (4, 5), and the paired-DRD technique permits calculation of the change (positive or negative) in total body vitamin A pool size that occurs in response to supplementation with different doses of vitamin A. The mean change in vitamin A pool size can be estimated as post-supplementation pool size minus initial pool size. To estimate the average daily vitamin A requirement, we are planning to use the paired-DRD technique to estimate the amount of daily vitamin A intake that is required to maintain a constant total body vitamin A pool size. The level of daily intake at which vitamin A pool size is maintained will be used as an estimate of the average daily vitamin A requirement, as described in detail below.

Hume and Krebs (1949) estimated the daily vitamin A requirement based on reversal of abnormal dark adaptation and normalization of plasma retinol concentrations in vitamin A-depleted subjects. Although the subjects were depleted of vitamin A over a period of ~ 1-2 years, only 3 of 16 subjects had changes in dark adaptation that were of sufficient magnitude to serve as an indicator of the curative effect of supplementation with vitamin A. One of those subjects was repleted with retinyl palmitate, and the other two were repleted with different levels of beta-carotene. A dose of 390 μg RE/d as retinyl palmitate for ~6 months resulted in normalization of dark adaptation, but the subject's plasma retinol concentration increased only slightly, from 17 μg/dL to 21 μg/dL. When the daily dose of vitamin A was raised to 780 μg/d for a period of 45 days there was no further improvement in the plasma retinol concentration. Thereafter, the subject was given a large daily dose of 7,200 μg/d and his plasma retinol concentration returned to his initial value of 33 μg/dL, and higher. Because he was not given any daily doses of vitamin A

between 780 μg/d and 7,200 μg/d, the level of daily intake at which his serum retinol concentration would have normalized is not known. Based on results from this single subject, it was concluded that the minimum daily vitamin A requirement was ~390 μg/d, and that a daily intake of 750 μg/d should meet the vitamin A needs of an adult human.

Sauberlich et al (1974) estimated the vitamin A requirement in eight men based on the amount of daily vitamin A intake required to correct impaired dark adaptation and abnormal electroretinograms, and to normalize plasma retinol concentrations. Additionally, the subjects received a tracer dose of ^{14}C-retinyl acetate to estimate the amount of vitamin A that is utilized daily during the depletion phase of the study. This vitamin A utilization rate was used as a second method to estimate the subjects' vitamin A requirement. During the depletion phase, impaired dark adaptation, abnormal electroretinograms and follicular hyperkeratosis were all associated with plasma retinol concentrations <30 μg/dL. Thus, a plasma retinol concentration >30 μg/dL was considered necessary to prevent the onset of clinical symptoms of deficiency in adult males. During the repletion phase, a daily intake of 150-300 μg/d corrected impaired dark adaptation, and 600 μg/d resulted in complete normalization of the electroretinogram in one of the eight subjects, and partial improvement in two others. However, skin lesions continued to manifest at this level of intake, and plasma retinol concentrations increased to >20 μg/dL, but not to >30 μg/dL. Thereafter, the subjects received a daily intake of 1200 μg/d which achieved plasma retinol concentrations >30 μg/dL in all subjects. Because plasma retinol concentrations >30 μg/dL were considered necessary for preventing the onset of clinical symptoms of deficiency, the investigators assessed the daily vitamin A utilization rate after 103 days of depletion when plasma retinol concentrations ranged from 29-34 μg/dl. At that point, the average daily vitamin A utilization rate was estimated as 910 μg/d, however the variability of the estimate was very high (range 570-1250 μg/d), and a standard deviation was not reported for the average utilization rate. Nevertheless, based the observed reversal of clinical symptoms of deficiency and the estimate of the amount of vitamin A utilized daily in these 8 subjects, it was concluded that the minimum daily vitamin requirement was ~600 μg/d, and that an intake of ~900 μg/d would maintain a plasma retinol concentration of ~30 μg/dL, and provide a modest reserve of vitamin A.

Thus, to date, there are very limited and highly variable data on which to estimate the vitamin A requirement. Moreover, the data that exist were based on a small number of men who were depleted of vitamin A and had small vitamin A pool sizes at the time that the requirement was estimated. Thus, there is no information available on the amount of daily vitamin A intake required to maintain higher vitamin A pool sizes. Currently, the recommended daily allowance for vitamin A in the US is set at 1000 μg/d for adult men, and is based on the limited data from the two depletion-repletion studies described above. The RDA for women and older children are extrapolated from the value for adult men, on the basis of body weight (RDA, 1989).

Because stable isotope techniques are now available for studying vitamin A metabolism safely in humans, we are proposing to use the paired-deuterated retinol dilution technique to estimate the average vitamin A requirement of adult males. We have done a series of studies in adults in Bangladesh using the deuterated retinol dilution technique to 1) study plasma retinol kinetics, 2) estimate total body stores of vitamin A, 3) detect changes in vitamin A stores in response to daily supplementation with known amounts of vitamin A, and 4) estimate bioconversion factors for beta-carotene to retinol from green leafy vegetables and sweet potatoes. Collectively, these studies have demonstrated that the deuterated retinol dilution technique provides an accurate estimate of total body vitamin A stores for groups of subjects (5), and that the technique can be used to assess differential changes in mean vitamin A pool sizes in response

to supplementation with vitamin A or beta-carotene (6, 7). Specifically, we estimated hepatic vitamin A stores of adult surgical patients (n=31) in Bangladesh using the DRD technique and by liver biopsy (5). Estimated mean hepatic vitamin A stores were similar by both techniques 0.110 $\pm$ 0.072 mmol (by DRD) and 0.100 $\pm$ 0.067 mmol (by biopsy), indicating that the technique provides a good estimate of vitamin A stores for groups of subjects. Additionally, we demonstrated that the DRD technique detected significant differences in final mean vitamin A pool sizes of three groups of subjects who received daily doses of either 0, 1.5 or 3.0 mg/d over a period of 75 or 129 days. The estimated final mean vitamin A pool sizes, as estimated by the DRD technique, were 0.048 $\pm$ 0.031, 0.252 $\pm$ 0.045 and 0.489 $\pm$ 0.066 mmol, ($p<0.001$) in the groups that received 0, 1.5 or 3.0 mg/d, respectively. Although initial pool size was not estimated directly in this study, the estimated mean change in vitamin A pool size was estimated as the final mean pool sizes of the supplemented groups minus the final mean pool size of the placebo group. Using this procedure, change in pool size for the groups receiving 1.5 or 3.0 mg/d were 0.204 mmol (95% CI:0.180, 0.228) and 0.440 mmol (95% CI:0.376, 0.505), ($p<0.001$). To determine whether the estimated mean changes in vitamin A pool size were reasonable, we estimated the expected change in vitamin A pool size using the theoretical assumptions that 50% of the supplemental vitamin A is retained (2, 8), and that the fractional catabolic rate for vitamin A in adults is 0.5%/d of the total body vitamin A pool (2, 9). Using these assumptions, the expected changes in vitamin A pool size in response to supplementation with 1.5 or 3.0 mg/d would be 0.189 mmol and 0.377 mmol, respectively. Thus, the estimated changes in vitamin A pool sizes, detected by the DRD technique, were similar to the expected theoretical changes. The ratio of estimated to theoretical change in pool size was 1.08 (95% CI: 0.82, 1.33) and 1.17 (95% CI: 1.00, 1.34) for groups that received 1.5 or 3.0 mg/d, respectively, (Table 1). In our most recent study on carotenoid bioavailability, we used the paired DRD technique to estimate change in vitamin A pool size in subjects who received 0.75 mg RE/d of vitamin A as either retinyl palmitate, beta-carotene in oil, beta-carotene from Indian spinach or sweet potatoes, or a placebo capsule for a period of 60 days. Because both initial and post-supplementation pool sizes were estimated, we were able to estimate change in vitamin A pool size as post-supplementation pool size minus initial pool size. In the group that received vitamin A palmitate, the estimated mean change in vitamin A pool size was 18.8 mg, by the paired DRD technique, and the expected change in pool size based on theoretical calculations was 18.5 mg (Table 1). Thus, the combined sets of data from these two studies indicate that DRD technique provides quantitative estimates of changes in vitamin A pool size that are very similar to the expected changes, based on theoretical calculations, especially at lower levels of daily intake (0.75 and 1.5 mg/d).

We are proposing to use the deuterated retinol dilution technique to estimate the average vitamin A requirement by estimating the amount of daily vitamin A intake that is required to maintain a constant vitamin A pool size. The paired-DRD technique will be used to assess change in vitamin A pool size in response to supplementation with increasing levels of daily vitamin A intake. Mean change in vitamin A pool size (post-supplementation pool size minus initial pool size) will be calculated for each level of dietary vitamin A intake (100-1000 µg/d). To estimate the average vitamin A requirement, regression analysis will be used to fit a line to the data on change in pool size vs. level of daily vitamin A intake. The point at which the regression line crosses the x-axis will be used as an estimate of the level of vitamin A intake at which vitamin A pool size is maintained. This level of daily intake will be used as an estimate of the amount of vitamin A intake required to maintain balance, which will be used as an estimate of the average daily requirement. A 95% confidence interval will be calculated for the regression line to obtain an estimate of the variation in the average daily requirement. There is currently no information on the variation of the vitamin A requirement. Thus, this information will be very useful for setting the recommended daily allowance for vitamin A.

The study will also estimate the amount of vitamin A required daily to maintain vitamin A pool size in subjects with higher initial vitamin A stores that are typical of US adults. Although the "optimal" vitamin A pool size for health is not known, an additional benefit of this study is that it will provide estimates of the amounts of daily vitamin A intake that are required to maintain low or high vitamin A pool sizes. Thus, if, at some point in the future, an "optimal" vitamin A pool size is identified based on specific functional outcomes, the results of this study will be useful in estimating the amount of daily vitamin A intake that would be required to maintain that pool size.

To illustrate the principle of the method we are proposing, we have pooled data on estimated change in vitamin A pool size in relation to daily vitamin A intake from two of our previous studies in Bangladeshi men to examine the relationship between change in vitamin A pool size and daily vitamin A intake. However, as previously described, initial pool size was not estimated directly in one study, so it was not possible to calculate change in pool size based on the difference between post-supplementation and initial pool sizes. For the purposes of this illustration, we estimated the mean change in vitamin A pool size for subjects in that study based on the assumption that their initial pool size would be similar to that observed for subjects in our most recent study on carotenoid bioavailability in which initial pool size was estimated directly. Thus, we estimated the change in vitamin A pool size of those subjects as their final estimated vitamin A pool size minus the mean initial pool size of subjects from the subsequent carotenoid bioavailability study. We feel that this assumption is acceptable because the subjects in both studies were recruited from the same population using the same selection criteria, and their baseline plasma retinol concentrations are similar (27.7 ± 5.4 μg/dL). As shown in Figure 1, when change in pool size is plotted against daily vitamin A intake, the relationship is linear. The regression line crosses the x-axis at a level of vitamin A intake of ~ 375 μg/d. Thus, based on these data, using this method, we would estimate that an average daily intake of ~375 μg/d is required to maintain a vitamin A pool size of ~30 mg (estimated initial mean vitamin A pool size of these subjects). In Figure 2, we have added the 95% confidence interval to the linear regression line shown in Figure 1. The levels of intake at which the 95% confidence interval crosses the x-axis are ~ 250 and 500 μg/d. Thus, based on these data, the estimated average amount of vitamin A intake required daily to maintain a pool size of ~30 mg would range from ~ 250 to 500 μg/d. Because these data span a wide range of daily vitamin A intakes (0-3000 μg/d), and because the 95% confidence interval is quite wide (250-500 μg/d), we are proposing to conduct a study to better define the regression line between the two variables to determine the level of intake that is required to maintain pool size. Specifically, we will study eight different levels of daily vitamin A intake (100-1000 μg/d) to define the regression line over a range of intakes that is very likely to include the average daily vitamin A requirement. Better definition of the regression line over this range of intakes will provide a more precise estimate of the point at which the regression line crosses the x-axis, which will be used as an estimate of the daily amount of vitamin A intake required to maintain pool size. Additionally, the 95% confidence interval for the regression line will be calculated to provide an estimate of variation in the average level of intake that is required to maintain vitamin A pool size. In addition, we will estimate the amount of daily vitamin A intake required to maintain higher vitamin A pool sizes that are typical of US adults. Because the data from experimental animals indicate that vitamin A disposal rates are higher when vitamin A pool sizes are large (10), we expect that higher amounts of daily intake will be required to maintain the vitamin A pool sizes that are typical of US adults, however that has not been demonstrated experimentally.

Research Design and Methods

Describe in detail the methods and procedures that will be used to accomplish the objectives and specific aims of the project. Discuss the alternative methods that are available and justify the use of the method proposed in the study. Justify the scientific validity of the methodological approach (biomedical, social, or environmental) as an investigation tool to achieve the specific aims. Discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Point out safety procedures to be observed for protection of individuals during any situations or materials that may be injurious to human health. The methodology section should be sufficiently descriptive to allow the reviewers to make valid and unambiguous assessment of the project

Study site: The study will be conducted at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), where we have conducted two previous studies with very similar designs, but different research questions. We have chosen to conduct the study in Bangladesh because our previous studies have demonstrated that it is possible to recruit men with low body stores of vitamin A based on their plasma retinol concentrations. Therefore, it is not necessary to deplete individuals of vitamin A to obtain a group of subjects with low initial vitamin A pool size (~30 mg). Also, because the traditional diet in Bangladesh is very similar to the low vitamin A basal diet that is used in the study, we have had excellent adherence to the dietary protocol in our previous studies.

Subjects:

Group 1: Healthy, Bangladeshi men (18-35 years of age) with assumed low total body stores of vitamin A (mean of ~30 mg), based on a plasma retinol concentration <35 μg/dL, will be selected for the study, as described in the next section. In our previous studies, Bangladeshi men with plasma retinol concentrations <35 μg/dL had an estimated mean total body vitamin A pool size of 26 ± 13.5 mg. Because their body weights averaged ~50 kg, their mean hepatic vitamin A concentration is estimated as ~ 22 μg/g (assuming liver weight is 2.4% of body weight (~1200 g) (9)), which is much lower than that of well-nourished adults in the US (~106 μg/g, (11)).

Group 2: Healthy, Bangladeshi men (18-35 years of age) with assumed low total body stores of vitamin A (mean of ~30 mg), will be selected, based on low plasma retinol concentrations, as described above. Following selection, they will be repleted with vitamin A such that their estimated mean total body stores of vitamin A will be ~130 mg, as described in the next section. This level of repletion was chosen to raise their liver vitamin A concentrations to levels typical of well-nourished North American men (~106 μg/g (11)).

The median liver vitamin A concentration of US adults, based on liver vitamin A concentrations at autopsy, is ~106 μg/g (11), which corresponds to a total body vitamin A pool size of ~ 180 mg, (assuming that liver is ~2.4% of body weight and average body wt is 70 kg). Although this level of hepatic vitamin A may be excessive for adequate vitamin A nutriture, it is common in the US, and is not associated with symptoms of vitamin A toxicity (3, 11). The amount of daily vitamin A intake required to maintain a pool size of ~180 mg is not known. Data from experimental animals indicate that the daily vitamin A disposal rate is higher in rats with larger vitamin A pool sizes (10). Although the mechanism that regulates the vitamin A disposal rate is not known, it is likely that the body catabolizes vitamin A at a higher rate when stores are larger to prevent accumulation of toxic levels of vitamin A in the liver. Thus, in humans, it is likely that larger daily intakes of vitamin A would be necessary to maintain vitamin A pool sizes of ~180 mg than would be needed to maintain smaller pool sizes. Based on theoretical

calculations, assuming 50% retention of vitamin A and a fractional catabolic rate of 0.5%/d of the body pool, a daily vitamin A intake of ~1,800 μg/d should be sufficient to maintain a vitamin A pool size of ~180 mg. However, this has not been demonstrated experimentally in humans. The mean daily vitamin A intake among US men was reported as ~ 1,163 ± 1,768 μg/d in NHANES III. Daily intakes of ~1800 μg/d are probably not unusual among US men because 49% of men over 20 years of age reported taking a daily multiple vitamin supplement, and the over-the-counter multiple-vitamin supplements generally contain 1.5 mg RE of vitamin A. Adults who consume a daily multiple-vitamin supplement and consume vitamin A-rich or vitamin A-fortified foods could easily reach daily intakes of ~1800 μg/d. Thus, in addition to estimating the level of daily vitamin A intake that is required to maintain pool size in subjects with smaller initial pool size (mean ~30 mg), we will also estimate the level of daily vitamin A intake required to maintain pool size in subjects with larger initial pool sizes (mean of ~130 mg; a liver vitamin A concentration of ~106 μg/g corresponds to a total vitamin A pool size of ~130 mg in adult Bangladeshi men and ~180 mg in US adults because of the difference in their body weights).

Subjects in group 2 (large initial pool size) will be repleted with vitamin A by administering four oral doses of 60 mg RE vitamin A palmitate 5 days apart, beginning 35 days prior to beginning the study procedures (study days -35, -30, -25, and –20). We expect that the average initial vitamin A pool size of the subjects will be ~30 mg. Based on this pool size, and the theoretical assumptions of 50% retention of vitamin A and a fractional catabolic rate of 0.5%/d of the total body vitamin A pool, we have calculated that the mean vitamin A pool size of these subjects should be ~130 mg at the onset of the study procedures (20 d after administration of the last high-dose supplement). It is necessary to wait 20 days following administration of the last vitamin A supplement to allow time for that dose to mix with the total body vitamin A pool.

We believe this repletion approach for obtaining a group with larger initial stores is acceptable because from our previous studies it is clear that Bangladeshi subjects respond, as expected, to supplementation with vitamin A. It is very unlikely that the Bangladeshi subjects that we have studied in the past have small vitamin A pool sizes because of genetic differences in vitamin A metabolism. In our previous studies, the estimated mean vitamin A pool sizes in the study populations increased, as expected, in response to supplementation with relatively low to high daily doses of vitamin A (0.75, 1.5 or 3.0 mg/d). And, as previously discussed, the mean increases in pool sizes were very similar to the expected increases based on theoretical assumptions of vitamin A retention and catabolism (table 1) (6, 7). Thus, the repletion protocol described above should result in a group of subjects mean total body vitamin A stores of ~ 130 mg.

Study procedures:

Initial screening: The study will be conducted in two rounds, because of space limitations at the study facility, with 32 participants in each round. For each round, 75 healthy, Bangladeshi men will be screened for plasma retinol and plasma CRP concentrations. Men with retinol concentrations <35 μg/dL and normal CRP concentrations will be eligible to participate in the study. Of those eligible, 32 with the lowest plasma retinol concentrations will be selected. The first group of 32 subjects will be assigned to treatment group 1, and they will complete the study protocol over a 4 month period during year 1. The following year a second group of 32 subjects will be selected and assigned to treatment group 2, and they will complete the study protocol over the same 4-month period during year two.

In round 1 (low initial vitamin A pool size), subjects will be randomly assigned to 1 of 8 treatment groups (n=4/group) to receive either 100, 200, 300, 400, 500, 600, 800 or 1000 μg RE/d as retinyl palmitate dissolved in corn oil in capsule form. The doses will be provided as two capsules daily, which will be administered to subjects with their noon and evening meals for a period of 60 days. The study physician will examine subjects for symptoms of vitamin A deficiency or other health problems weekly. In our previous studies, we have administered 0 mg/d for a period of 60 days and have not observed any symptoms or signs of vitamin A deficiency in these subjects. In the present study, the lowest level of supplementation will be 100 μg/d; thus, we do not anticipate any symptoms of deficiency in these subjects.

In round 2, (large initial vitamin A pool size), subjects will be randomly assigned to 1 of 8 treatment groups (n-4/group) to receive either 200, 500, 800, 1100, 1400, 1700, 2000, or 2300 μg RE/d as retinyl palmitate dissolved in corn oil in capsule form. The capsules will be administered with their noon and evening meals, as described above for round 1. The upper level of supplementation for this group is 2300 μg RE/d, which is less than the Upper Tolerable Limit of intake of 3000 μg RE/d recommended by the Institute of Medicine (3).

During the supplementation phase of the study, subjects will receive a low vitamin A basal diet, supplemented with the varying levels of vitamin A in capsule form. The minimal amount of vitamin A provided by the diet (very small amounts of provitamin A carotenoids from pale colored fruits/vegs (banana, cucumber, cabbage) ~75-100 μg RE/d) will be measured and included in the final estimate of vitamin A intake for all of the treatment groups. Thus, including the estimated vitamin A from the basal diet, daily intakes will range from ~175 μg/d to 2400 μg/d for a period of 60 days.

Study design

A diagram of the study design is presented in Figure 3.

Initial vitamin A pool size estimate:

Subjects will begin consuming the low vitamin A basal diet 3 days prior to receiving an oral dose of 10 mg of [$^2H_8$]-retinyl acetate for estimation of initial pool size, and will continue to receive the low vitamin A diet for 21 days. (Because subjects in group 2 will have larger vitamin A pool sizes, they will receive a dose of 15 mg of [$^2H_8$]-retinyl acetate. This will result in plasma isotopic ratios of labeled to non-labeled vitamin A of ~5% in subjects with pool sizes of ~130 mg.) Blood samples will be drawn 20 and 21 days after administration of the isotope for measurement of the plasma isotopic ratio of [$^2H_8$]-retinol:retinol and estimation of initial vitamin A pool size. It is necessary to keep the subjects on the low vitamin A diet until blood is drawn for estimation of the vitamin A pool size because newly absorbed dietary vitamin A is preferentially secreted into the plasma and can affect the plasma isotopic ratio of labeled to non-labeled vitamin A. In a previous study, we found that vitamin A pool size is overestimated when dietary vitamin A intake is high during the period in which pool size is estimated, because the unlabeled vitamin A coming from the diet dilutes the labeled vitamin A in the plasma pool resulting in a lower plasma isotopic ratio of labeled to non-labeled vitamin A and overestimation of the vitamin A pool size. For that reason, we plan to keep the subjects on the low vitamin A basal diet when estimating vitamin A pool size by the DRD technique.

The plasma retinol concentration will also be measured in the day 21 sample.

Supplementation phase:

On day 22, subjects will begin receiving their assigned dose of vitamin A daily with their noon and evening meals (2 capsules/d), for a period of 60 days. Blood samples will be drawn following 60 days of supplementation for measurement of the plasma retinol concentration to determine whether plasma retinol responds to the different levels of dietary vitamin A intake.

Stabilization period:

Following the supplementation period, subjects will consume the low vitamin A basal diet for a period of 15 days to allow time for the vitamin A that was provided during the supplementation phase to mix with the vitamin A body pool.

Post-supplementation vitamin A pool size estimate:

Immediately following the 15-day stabilization period, subjects will receive an oral dose of 10 mg [$^2H_4$]-retinyl acetate (group 1) or 15 mg [$^2H_4$]-retinyl acetate (group 2) for estimation of the post-supplementation pool size. We will use a different isotope, [$^2H_4$]-retinyl acetate, rather than [$^2H_8$]-retinyl acetate, for the final pool size determination so that residual [$^2H_8$]-retinol in the plasma from the first dose of isotope will not interfere with the measurement of the of the post-supplementation plasma isotopic ratio and estimation of final pool size. Blood samples will be drawn on days 20 and 21 for measurement of the plasma isotopic ratio and estimation of vitamin A pool size. During these 21 days the subjects will continue to consume the low vitamin A basal diet. At the end of the study all subjects in group 1 (low vitamin A body stores) will receive a single high dose of vitamin A (60 mg RE).

Blood collection schedule:

The total vitamin A body store (or 'pool size') will be measured twice in each subject – before and after the 60-day supplementation – with various doses of vitamin A as mentioned above. To estimate vitamin A pool size, the subjects will receive an oral dose of vitamin A labeled with non-radioactive stable isotope, [$^2H_4$]-retinyl acetate. 10 ml of blood will be drawn before administering the labeled vitamin A and then 1, 3, 20 and 21 days after giving the dose to measure plasma isotopic ratios of labeled to non-labeled vitamin A. In addition, 10 ml of blood will be drawn just after the end of supplementation period to measure plasma retinol concentration. Thus, blood will be collected from each subject for 10 times (day 1, 3, 20, 21, 83, 94, 95, 97, 114, and 115) during the study period as shown in Fig 3 (Appendix-3).

Laboratory Analyses:

The isotopic ratio of [$^2H_4$]-retinol/retinol in plasma will be determined by GC-MS as first described Clifford et al (12) and modified by Handelman et al (13). Briefly, retinol will be isolated from plasma by HPLC, and the tert-butyldimethylsilyl (tBDMS) derivative of 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 ([$^2H_4$]-retinol). A set of calibration standards with of [$^2H_4$]-retinol/retinol weight ratios of 0.00, 0.0167, 0.050, 0.167 and 0.50 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 m/z 255. The area ratios for the plasma samples will be

substituted into the regression equation to solve for 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 [$^2H_8$]-retinol/retinol will be measured using the same method. The plasma retinol concentration will be determined by HPLC as previously described (14).

Sample size:

At least 3 treatment groups (levels of vitamin A intake) are needed to detect significant departures from linearity of the relationship between change in pool size and level of daily vitamin A intake, and at least 2 subjects per treatment group are needed to determine whether a given equation provides good fit. To better define the curve, we have chosen to include 8 levels of intake, with 4 subjects in each treatment group. This will allow us to estimate more precisely the level of intake that is associated with maintaining vitamin A pool size and the variability associated with that estimate. According to Neter, et al (15), an approximate confidence interval for the point at which the regression line $y = a + b * x$ crosses the x-axis can be calculated from the mean square error of the regression, the estimated slope, the total sample size, and the distance of the estimated value of the dose from the mean value of the dose. According to our calculations, this suggests that we will be able to estimate the maintenance dose within 125 μg for group 1 and within 150 μg for group 2.

Expected outcomes:

Using the theoretical assumptions of vitamin A retention (50%) and the fractional catabolic rate of vitamin A (0.5%/d of body pool), it is possible to estimate the expected change in vitamin A pool size in response to supplementation with increasing levels of vitamin A over a given time period. Figure 4 shows an approximation of the expected results from round 1 of the study, using this approach. Assuming that subjects have a mean initial vitamin A pool size of ~30 mg, the change in pool size in response to supplementation will range from ~ -6.5 mg at an intake level of 100 μg/d to ~15.6 mg at an intake of 1000 μg/d. We believe that these estimates are reasonable because in our previous studies, estimates of change in pool size based on the same theoretical calculations have been very similar to the actual estimates of change in pool size that we obtained in the using the DRD technique (see table 1). As shown in Figure 4, the point at which the regression line crosses the x-axis is ~375 μg/d. Based on these estimates, a daily intake of ~375 μg/d would be necessary to maintain a pool size of ~30 mg.

Using the same approach, we have estimated the expected results from round 2 (high vitamin A pool size) of the study (figure 5). Assuming that subjects have a mean initial vitamin A pool size of ~130 mg, the change in pool size in response to supplementation will range from ~ -33.5 mg at an intake level of 200 μg/d to ~18.2 mg at an intake of 2300 μg/d. Based on these estimates, a daily vitamin A intake of ~1600 μg/d would be necessary to maintain a pool size of ~130 mg.

Pitfalls and limitations:

Adherence to study protocol: It is possible that subjects will consume vitamin A-containing foods during the evening when they are not under supervision. This is very unlikely because their diets are typically low in vitamin A, so even if they did eat or drink something away from the study center, it is not likely to contain vitamin A. Also, in our previous studies we have

not had any indication that consumption of vitamin A-rich foods occurred outside of the study facility. The estimated pool sizes of the placebo group have always declined, as expected, in response to the low vitamin A diet.

Analytical techniques:

There may be some concern that the GC-MS technique is not sensitive enough to detect changes in vitamin A pool size in response to supplementation with small doses of vitamin A. In our recent study on carotenoid bioavailability, we detected differences of –6.5 mg, 1.4 mg, 6.4 and 12.3 mg in mean vitamin A pool sizes across treatment groups. In the proposed study we are expecting to find differences in mean pool sizes ranging from -6.5 mg to 15.6 mg (range=18.8 mg) for subjects with low vitamin A pool size, which are very similar to the mean changes in pool size that were estimated using the DRD technique in our carotenoid bioavailability study. For subjects with high initial pool size, we are expecting to find differences in pool size ranging from ~ –33.5 mg to 18.2 mg (range=52 mg). Thus, we do not anticipate any problem in detecting changes in vitamin A pool sizes in response to the amounts of daily vitamin A that the subjects will be receiving. Additionally, we are constructing a curve rather than attempting to detect significant differences in mean pool sizes across treatment groups. With eight increasing levels of daily vitamin A intake, over a range of 100-1000 μg/d (group 1), or 200-2300 μg/d (group 2), we will obtain data points over a sufficiently wide range of intakes to obtain a very good estimate of the regression line, which will then allow us to estimate the level of daily vitamin A intake required to maintain a constant pool size.

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 the laboratory facilities and major equipment that will be required for the study. For field studies, describe the field area including its 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 (model 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 professionals. Specify what statistical software packages will be used and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to monitor further progress of the study. (TYPE WITHIN THE PROVIDED SPACE).

Vitamin A pool size will be estimated using the measured plasma isotopic ratios of labeled to non-labeled vitamin A at 20 days post-dose, and the isotope dilution equation for estimate of pool size (0.5 * dose (0.65 $e^{-0.005 \text{ (days since dose)}}$ (H:D-1)) (4). The assumptions of the equation are that 50% of the dose is retained, the ratio of labeled to non-labeled vitamin A in plasma to liver is 0.65, and that the fractional catabolic rate is 0.5%/d of the total body pool. Although these assumptions can be challenged, in practice, when compared with direct measurement of vitamin A in liver biopsy specimens, and when compared with theoretical estimates of expected change in pool size in response to supplementation with known amounts of vitamin A, the equation provides a good estimate to total body vitamin A pool size for groups of subjects (5, 6), as previously described. The mean change in pool size (post-supplementation pool size minus initial pool size) will be estimated, controlling for initial values, for each level of dietary vitamin A intake. The level of intake at which vitamin A pool size is maintained will be estimated by fitting a straight line or curve (as appropriate) to the observed data on change in pool size vs level of daily vitamin A intake and estimating the point at which the curve crosses the x-axis. (For theoretical reasons, we believe that a straight line will describe the relationship, but if there is a significant departure from linearity, we will test other models). The point at which the curve crosses the x-axis will be assumed to represent the level of daily vitamin A intake required to maintain pool size, and will serve as an estimate of the average vitamin A requirement.

The curves for group 1 and group 2 will be tested to see if they differ significantly from each other (e.g., linear relationships will be tested for group effect and group by dose interaction using analysis of covariance including a test for equal slopes). Although we expect that the curves will be different, if they do not differ at the 10% level of significance, the data from both groups of subjects will be pooled to estimate the level of intake at which vitamin A body stores are maintained. If the curves are different, as expected, separate curves will be estimated for both groups of subjects (small and large initial body stores).

Mean change in plasma retinol concentrations (post-supplementation minus initial values) will be examined across treatment groups using analysis of covariance, controlling for initial values.

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 on sick individuals, provide sufficient reasons for using them. Indicate how subject's rights are protected and if there is any benefit or 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. "Heavy vitamin A" is a non-toxic substance that poses no risk to an individual. Removing blood from the vein of the subjects may cause some discomfort, bruising, dizziness and rarely infection. The subjects will be administered vitamin A capsules daily for a period of 60 days – the dosage will vary from 100 – 2300 µg/day according to random distribution as described in the Methods of the protocol. Thus, some of the subjects will receive less than the current recommended daily allowance of 700 µg/d for adult males. These subjects, however, will receive a total of 175-200 µg/d from the diet and capsules combined. It is very unlikely that clinical signs of vitamin A deficiency will occur at this level of intake. Upon completion of the study, they will be given a single, high-dose vitamin A supplement (60 mg retinyl palmitate) to replete their vitamin A stores. Some of the subjects, on the other hand, will be receiving as high as 2300 µg/d of vitamin A capsules which is still below the Upper Limit (3000 µg/d) established by the Institute of Medicine. 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 at any time after the commencement of the study. 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 as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however exercise judgment in assessing the "standard" length.

1. Hume EM, Krebs HA. (1949) Vitamin A Requirement of Human Adults. Med. Res. Council. Special Report #264,
Her Majesty's Stationery Office, London;264:1-145.

2. Sauberlich HE, Hodges HE, Wallace DL, et al. (1974) Vitamin A metabolism and requirements in the human studied
with the use of labelled retinol. Vitam Horm;32:251-75.

3. Institute of Medicine. Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. (2001) . Washington, DC: National Academy Press.

4. Furr HC, Amedee-Manesme O, Clifford AJ, Bergen RH, Jones AD, Anderson DP, Olson JA (1989) Vitamin A concentrations in liver determined by isotope dilution assay with tetradeuterated vitamin A and by biopsy in generally healthy adult humans. Am J Clin Nutr;49:713-16.

5. Haskell MJ, Handelman GJ, Peerson JM, Rabbi A, Awal MA, Wahed MA, Mahalanabis D, Brown KH. (1997) 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;66:67-74.

6. Haskell M, Mazumder R, Peerson J, Wahed MA, Mahalanabis D, Brown KH. (1999) Use of the deuterated retinol dilution (DRD) technique to assess total body vitamin A stores of adult volunteers consuming different levels of vitamin A. Am J Clin Nutr;70:874-80.

7. Haskell M, Jamil K, Peerson J, Wahed MA, Fuchs G, Brown KH. Response of total body stores of vitamin A to daily supplementation with green leafy vegetables or sweet potato in Bangladeshi men. (2001) International Congress of Nutrition. Vienna, Austria.

8. Bausch J, Rietz P. (1977) Method for the assessment of vitamin A liver stores. Acta Vitaminol Enzymol;31:99-112.

9. Olson JA. (1987) Recommended dietary intakes (RDI) of vitamin A in humans. Am J Clin Nutr;45:704-16.

10. Green MH, Green JB, Lewis KC. (1987) Variation in retinol utilization rate with vitamin A status in the rat. J Nutr;117:694-703.

11. Raica NJ, Scott BS, Lowry L, Sauberlich HE. (1972) Vitamin A concentration in human tissues collected from five areas in the United States. Am J Clin Nutr;25:291-296.

12. Clifford AJ, Jones AD, Tondeur Y, Furr HC, Bergen HR, Olson JA. (1986) Assessment of vitamin A status of humans by isotope dilution GC/MS. Proc Mass Spectrom Allied Top;34:327-8.

13. Handelman GJ, Haskell MJ, Jones AD, Clifford AJ. (1993) An improved protocol for determining ratios of retinol-d4 to retinol isolated from human plasma. Analytical Chemistry;65:2024-2028.

14. Bieri JG, Tolliver TJ, Catignani GL. (1979) Simultaneous determination of alpha-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. Am J Clin Nutr;32:2143-2149.

15. Neter J, Wasserman W, Kutner M. Applied Linear Statistical Models, 2nd edition. (1985). Homewood, Illinois: : Richard D. Irwin, Inc.

Dissemination and Use of Findings

Describe explicitly the plans for disseminating the accomplished results. Describe what type of publication is anticipated: working papers, internal (institutional) publication, international publications, international conferences and agencies, workshops etc. Mention if the project is linked to the Government of Bangladesh 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 presented elsewhere including workshops, seminars, etc. as appropriate. Eventually the paper will be submitted for publication in a peer-reviewed journal on nutrition.

Collaborative Arrangements

Describe briefly if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between 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 Centre for Diarrhoeal Disease Research, Bangladesh (David Sack, Director).

Biography of the Investigators

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

1 Name : Kazi Mohammad Asif Jamil

2 Present position : Senior Medical Officer, Grade I

3 Educational background (last degree and diploma & training relevant to the present research proposal):

1988: M.B.B.S. (Bachelor of Medicine and Surgery)
Institute – Chittagong Medical College, Chittagong, Bangladesh

1995: Ph.D. in Medical Sciences
Institute – University of Tokyo, Japan

2001: Postdoctoral training in Nutrition at the Department of Nutrition of University of California Davis, USA

4 List of ongoing research protocols
(start and end dates; and percentage of time)

4.1. As Principal Investigator

Protocol Number	Starting date	End date	Percentage of time
2001-028	1-3-02	28-2-03	20

4.2. As Co-Principal Investigator

Protocol Number	Starting date	End date	Percentage of time

4.3. As Co-Investigator

Protocol Number	Starting date	Ending date	Percentage of time

5 Publications

Types of publications	Numbers
a) Original scientific papers in peer-review journals	3
b) Peer reviewed articles and book chapters	
c) Papers in conference proceedings	
c) Letters, editorials, annotations, and abstracts in peer-reviewed journals	3
d) Working papers	
b) Monographs	

6 Recent publications including publications relevant to the present research protocol

1) Response of estimated total body vitamin A stores to daily supplementation with green leafy vegetables or sweet potatoes in healthy Bangladeshi men. Haskell MJ, **Jamil KM**, Peerson J, Wahed MA, Fuchs GJ, Brown KH. (2001) FASEB Journal 15: 228.6 (Abstract)
2) Low temperature hemodialysis prevents hypotensive episodes by reducing nitric oxide synthesis. **Jamil KM**, Yokoyama K, Takemoto F, Hara S, and Yamada A. Nephron. 2000 Mar;84(3):284-6.
3) Distinct mechanisms of action of V1 antagonists OPC-21268 and [d(CH_2)$_5$Tyr(Me)AVP] in mesangial cells. **Jamil KM**, Watanabe T, Nakao A, Okuda T and Kurokawa K. Biochem. Biophys. Res. Commun. 1993; 193: 738-743.
4) Expression of platelet activating factor receptor in renal tubular cell line (LLC-PK1). **Jamil KM**, Takano T, Nakao A, Honda Z, Shimizu T, Watanabe T and Kurokawa K. Biochem. Biophys. Res.Commun. 1992; 187: 767-772.

Marjorie J. Haskell

Curriculum Vitae

Program in International Nutrition, Dept. of Nutrition
University of California-Davis, Davis, CA 95616
(W) (530) 752-1992 (H) (530) 757-6326
612 Hudson Street, Davis, CA 95616

EDUCATION

1996	Ph.D. in Nutrition with Designated Emphasis in International Nutrition University of California-Davis Davis, CA
1987	M.P.H. in Nutrition University of North Carolina Chapel Hill, NC
1985	B.S. in Nutrition University of Massachusetts Amherst, MA
1980	B.A. in French Western Kentucky University Bowling Green, KY
1978-79	French language study Universite de Montpellier Montpellier, France

PROFESSIONAL AND RESEARCH EXPERIENCE

July 2000 to present	**Assistant Nutritionist, Prog. in International** Nutrition, Nutrition Department University of California, Davis, Davis, CA.
July 1996 to June 2000	Post-doctoral Researcher, Prog. in International Nutrition, Nutrition Department University of California, Davis, Davis, CA.
November 1997 to Feb 1998	Consultant, Market Access for Rural Development, Dang, Nepal. Assessed food and nutrition beliefs and practices of women with young children in the mid-western Terai in Nepal.
Nov-Dec 1996; July-October 1995	Consultant, International Atomic Energy Agency, Vienna, Austria. Assisted with implementation of pilot studies to assess vitamin A status of infants and school-aged children in Peru, using stable isotope labeled vitamin A.
September 1992 to June 1996	Post-graduate researcher, Program in International Nutrition, Nutrition Department, University of California, Davis, Davis, CA. Completed dissertation project entitled, "Assessment of vitamin A status in humans using the deuterated retinol dilution technique".
September 1992 to	Conducted clinical phase of dissertation project

December 1993	at the International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh.
September 1991 to August 1992	Post-graduate researcher, Nutrition Department, University of California Davis, Davis, CA. Analyzed human plasma, breastmilk, and foods for vitamin A and carotenoid content by HPLC.
September 1991 to June 1992	Teaching Assistant, Nutrition Department, University of California Davis, Davis, CA. Assisted in teaching two undergraduate courses on diet therapy, and a course on nutritional assessment (anthropometry, body composition, and assessment of dietary intake).
February 1989 to September 1990	Research Assistant, Center for Studies of Sensory Impairment, Aging and Metabolism, Guatemala City, Guatemala. Managed a field study to assess the nutritional status of Guatemalan elderly living in a peri-urban neighborhood of Guatemala City.
June 1987 to June 1988	Research Assistant, Vitamin Bioavailability Laboratory, USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA. Studied the intestinal uptake of folic acid, and measured the folate content of foods by HPLC.
January 1986 to April 1986	Dietetic Intern, Duke University Medical Center, Durham, North Carolina. Completed a dietetic internship in fulfillment of the requirements of the American Dietetic Association for registration as a dietitian. Completed clinical requirements in gastroenterology, endocrinology, cancer, obstetrics, and eating disorders.
February 1985 to August 1985 (and Summer 1986)	Laboratory Technician, Nutrition Evaluation Laboratory, USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA. Performed assays for analyzing human blood samples for various nutrients, including vitamins A and E by HPLC, and enzymatic assays for vitamins B1, B2 and B6.

PROFESSIONAL AFFILIATIONS
Member of American Society for Nutritional Sciences
Member of the Society for International Nutrition Research
RD eligible

AWARDS AND HONORS
Finalist, Student competition, Society for International Nutrition Research, 1996
ARCS scholarship, 1992
Out-state-tuition waiver, University of California, Davis, 1990

LANGUAGES: Fluent in Spanish, limited fluency in French

REFERENCES: Available on request.

PUBLICATIONS

Haskell MJ, Lembcke JL, Salazar M, Green MH, Peerson JM and Brown, KH. Population-based plasma kinetics of an oral dose of [$^2H_4$]-retinyl acetate among preschool aged Peruvian children, AJCN, in press, 2002.

Allen LH, Haskell MJ. Estimating the potential for vitamin A toxicity in women and young children. J of Nutrition, in press, 2002.

Allen LH, Haskell MJ. Vitamin A requirements of infants under six months of age. Food Nutr. Bull, 22:214-234, 2001.

Haskell MJ, Mazumder RN, Jones AD, Peerson JM, Wahed MA, Mahalanabis D, and Brown KH. Use of the deuterated retinol dilution technique to assess total body vitamin A reserves of adult volunteers consuming different levels of vitamin A. Am J Clin Nutr, 70:874-80, 1999.

Haskell MJ, Brown KH. Maternal vitamin A nutriture and the vitamin A content of human milk. J of Mammary Gland Biology and Neoplasia, 4:243-257, 1999.

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, 68:90-95, 1998.

Haskell MJ, Handelman GJ, Peerson JM, Jones AD, Rabbi, A, Awal MA, Wahed MA, Mahalanabis D, and Brown K.H. 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, 66:67-74, 1997.

Manuscripts in preparation:

Haskell MJ, Jamil KMA, Hossain I, Peerson JM, Wahed MA, Fuchs G, Brown, KH. Response of total body stores of vitamin A in Bangladeshi men to daily supplementation with green leafy vegetables or sweet potatoes, 2002.

MJ Haskell, P Pandey, J Graham, JM Peerson, RK Shresthra and KH Brown. Response of

KH Brown, MJ Haskell, JM Peerson, P Pandey, JM Graham, and RK Shrestha Relationships among indicators of vitamin A status in nightblind and non-nightblind pregnant Nepali women, 2002.

Kenneth H. Brown, M.D.
Curriculum Vitae

Department of Nutrition, University of California, Davis, California 95616-8669
Telephone No. (916) 752-1992 FAX No. (916) 752-3406 E-Mail: KHBrown@UCDavis.edu (Internet)

Current Appointments
Professor, Department of Nutrition University of California, Davis, California
Director, Program in International Nutrition, University of California, Davis, California

Education

1964-1968	Columbia University, New York, New York - B.A.
1968-1973	University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania - M.D.

Training and Professional Experience

1973-1975	Internship & Assistant Residency in Pediatrics, Boston Children's Hospital Medical Center, Boston, MA (Clinical Fellow in Pediatrics, Harvard University School of Medicine)
1975-1977	Research Associate, Departments of Medicine and Pathology, Johns Hopkins University School of Medicine and Sch. of Hygiene and Public Health, Baltimore, MD (Visiting Scientist, Internat'l Center for Medical Research, and the Cholera Research Laboratory, Dhaka, Bangladesh)
1978-1984	Assistant Professor, Department of Pediatrics, Johns Hopkins University School of Medicine
1980-1985	Visiting Scientist, Instituto de Investigación Nutricional, Lima, Perú
1984-1989	Associate Professor, Dept. of Pediatrics and Dept. of Internal Health School of Hygiene & Pub Hlth, Johns Hopkins University School of Medicine
1985-1989	Director, Division of Human Nutrition, Department of International Health, Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD
1989-	Professor, Department of Nutrition, University of California, Davis, California
1989-	Director, Program in International Nutrition, University of California, Davis, California

Certification and Memberships
Certified American Board of Pediatrics, 1979; American Society for Clinical Nutrition, 1982; American Institute of Nutrition (AIN), 1982; Society for Pediatric Research, 1984; International Society for Research on Human Milk and Lactation, 1989; Society for International Nutrition Research, 1990; Commonwealth Association of Paediatric Gastroenterology and Nutrition, 2000

Selected Appointments

1979, 1986	World Health Organization, Diarrhoeal Disease Control Programme, Scientific Working Group on Drug Development
1984-1989	National Research Council, Food and Nutrition Board, International Nutrition. Program, Subcommittee on Nutrition and Diarrheal Diseases Control
1986-1989	U.S. National Committee for the International Union of Nutritional Sciences (USNC/IUNS)
1987-1989	National Research Council, Food and Nutrition Board, Committee on International Nutrition Programs
1990-1991	President-Elect, Society for International Nutrition Research
1992-1994	President, Society for International Nutrition Research
1993-1996	Councilor, The American Society for Clinical Nutrition
1994	Tech. Advisory Committee, Food and Nutrition Program, Pan American Health Organ.
1994-1995	Member, Committee on International Nutrition, National Academy of Science
2000	Member, Rice Fortification Technical Advisory Panel, Program for Applied Technical Health (Path)

2000 Editorial Board, Journal of Health, Population and Nutrition
2000 Chair, Steering Committee of the International Zinc Nutrition Consultative Group
2000 Member, Executive Committee of the International Society for Research on Human Milk and Lactation

Board Appointments

Editorial Boards

Editorial Board, Journal of Diarrhoeal Disease Research, 1984-1999; Editorial Board, European Journal of Clinical Nutrition, 1990-date; Assistant Editor, American Journal of Clinical Nutrition, 1991-1998; Editorial Committee, Revista de Salud Publica de Mexico, 1996-date; Nutrition Section Editor, Journal of Health, Population and Nutrition, 2000-date

Institutional Advisory Boards

International Advisory/Technical Group, Center for Studies of Sensory Impairment, Aging, and Metabolism (CeSSIAM), Guatemala City, Guatemala, 1985-1995; Scientific Advisory Committee, Instituto de Investigación Nutricional, Lima, Peru, 1989-date

Foreign Language Skills

Spanish -- Fluent speaking, excellent reading, fair writing
French, Bengali, Thai -- Limited working knowledge

Awards and Honors

1993 International Award for Modern Nutrition, Swiss Central Milk Producers Association, Bern, Switzerland
1995 Kellogg International Nutrition Research Prize, Society for International Nutrition Research
1997 McCollum Award, American Society for Clinical Nutrition

PUBLICATIONS: (recent papers selected from a total of more than 195 publications)

Brown KH, Sanchez-Griñan M, Perez F, Peerson JM, Ganoza L, Stern JS. Effects of dietary energy density and feeding frequency on total daily energy intakes by recovering malnourished children. Am J Clin Nutr 62:13-18, 1995.

Molina S, Vettorazzi C, Peerson JM, Solomons NW, Brown KH. Clinical trial of glucose-oral rehydration solution (ORS), rice dextrin-ORS, and rice flour-ORS for the management of children with acute diarrhea and mild or moderate dehydration. Pediatrics 92:191-197, 1995.

Dewey KG, Peerson JM, Brown KH, Krebs NF, Michaelsen KF, Persson LA, Salmenpera L, Whitehead RG, Yeung DL. Growth of breast-fed infants deviates from current reference data: a pooled analysis of US, Canadian, and European data sets. Pediatrics 96:495-503, 1995.

Brown KH, Peerson JM, Lopez de Romaña G, Creed de Kanashiro, Black RE. Validity and epidemiology of reported poor appetite among Peruvian infants from a low-income, periurban community. Am J Clin Nutr 61:26-32, 1995.

Piwoz EG, Creed de Kanashiro H, Lopez de Romaña G, Black RE, Brown KH. Potential for misclassification of infants' usual feeding practices using 24-hour dietary assessment method. J Nutr 125:57-65, 1995.

Lönnerdal B, Zavaleta N, Kusunoki L, Lanata CF, Peerson JM, Brown KH. Effect of postpartum maternal infection on proteins and trace elements in colostrum and early milk. Acta Paediatr 85:537-42, 1996.

Ashraf H, Bhan MK, Bhatnagar S, Bhutta Z, Brown KH, Dung PT, Fontaine O, Garcia Aranda JA, Issani Z, Ly DT, Mahalanabis D, Mitra AK, Molla AM, Nizami SQ, Nurko S, Penny M, Singh KD, Snyder JD, Thanh PN, Thobani S, Verne E. International Working Group on Persistent Diarrhoea. Evaluation of an algorithm for the treatment of persistent diarrhoea: a multicentre study. Bull World Health Organization, 74: 479-489, 1996.

Ruel MT, Rivera JA, Santizo M-C, Lönnerdal B, Brown KH. Impact of zinc supplementation on morbidity from diarrhea and respiratory infections among rural Guatemalan children. Pediatrics 99:808-813, 1997.

Dewey KG, Cohen RJ, Landa Rivera L, Canahuati J, Brown KH. Effects of age at introduction of complementary foods to breast-fed infants on duration of lactational amenorrhea in Honduran women. Am J Clin Nutr 65:1403-1409, 1997.

Brown, KH. Complementary feeding in developing countries: factors affecting energy intake. Proc Nutr Soc 56:139-148, 1997.

Ruel MT, Dewey KG, Martínez C, Flores R, Brown KH. Validation of single daytime samples of human milk to estimate the 24-h concentration of lipids in urban Guatemalan mothers. Am J Clin Nutr 65:439-44, 1997.

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

Lembcke JL, Peerson JM, Brown KH. Acceptability, safety, and digestibility of spray-dried bovine serum added to diets of recovering malnourished children. J Pediatr Gastroenterol Nutr 25:381-384, 1997.

Brown KH, Peerson JM, Allen LH. Effect of zinc supplementation on children's growth: A meta-analysis of intervention trials. Bibliotheca Nutritio et Dieta 54:76-83, 1998.

Rivera JA, Ruel MT, Santizo M-C, Brown KH, Lönnerdal B. Zinc supplementation improves the growth of stunted rural Guatemalan infants. J Nutr 128:556-562, 1998.

Haskell MJ, Islam MA, Handelman GJ, Peerson JM, Jones AD, Wahed MA, Mahalanabis D, Brown KH. Plasma kinetics of an oral dose of $^{2}H_{4}$-retinyl acetate in human subjects with low or high total body stores of vitamin A. Am J Clin Nutr 68:90-95, 1998.

Brown KH, Dewey KG, Allen LH. Complementary feeding of young children in developing countries: A review of current scientific knowledge. World Health Organization (WHO/NUT/98.1), Geneva, Switzerland, 1998.

Brown KH. Effect of infections on plasma zinc concentration and implications for zinc status assessment in low-income countries. Am J Clin Nutr 68:425S-429S, 1998.

Mendoza C, Viteri FE, Lonnerdal B, Young KA, Raboy V, Brown KH. Effect of genetically modified, low-phytate maize on absorption of iron from tortilla. Am J Clin Nutr 68:1123-1127, 1998.

Goto K, Chew F, Torun B, Peerson JM, Brown KH. Epidemiology of altered intestinal permeability to lactulose and mannitol in Guatemalan infants. J Pediatr Gastroenterol Nutr 28:282-290, 1999.

Bennett VA, Morales E, Gonzalez J, Peerson JM, Lopez de Romana G, Brown KH. Effects of dietary viscosity and energy density on total daily energy consumption by young Peruvian children. Am J Clin Nutr 70:285-191, 1999.

Penny ME, Peerson JM, Marin RM, Duran A, Lanata CF, Lönnerdal B, Black RE, Brown KH. Randomized, community-based trial of the effect of zinc supplementation, with and without other micronutrients, on the duration of persistent childhood diarrhea in Lima, Peru. J Pediatr 135:208-217, 1999.

Lartey A, Manu A, Brown KH, Peerson JM, Dewey KG. A randomized, community-based trial of the effects of improved, centrally processed complementary foods on growth and micronutrient status of Ghanaian infants from 6 to 12 mo of age. Am J Clin Nutr 70:391-404, 1999.

Haskell MJ, Mazumder RN, Peerson JM, Jones AD, Wahed MA, Mahalanabis D, Brown, KH. Use of the deuterated-retinol technique to assess total-body vitamin A stores of adult volunteers consuming different amounts of vitamin A. Am J Clin Nutr 70:874-880, 1999.

Dewey KG, Cohen RJ, Brown KH, Rivera LL. Age of introduction of complementary foods and growth of term, low-birth-weight, breast-fed infants: a randomized intervention study in Honduras. Am J Clin Nutr 69:679-86, 1999

Zinc Investigators' Collaborative Group (Bhutta ZA, Black RE, Brown KH, Meeks-Gardner J, Gore S, Hidayat A, Khatun F, Martorell R, Ninh NX, Penny ME, Rosado JL, Roy SK, Ruel M, Sazawal S, Shankar A). Prevention of diarrhea and pneumonia by zinc supplementation in children in developing countries: Pooled analysis of randomized controlled trials. J Pediatr 135: 689-97, 1999.

Lartey A, Manu A, Brown KH, Dewey KG. Predictors of micronutient status among six- to twelve-month-old breast-fed Ghanaian infants. J Nutr 130: 199-207, 2000.

Lartey A, Manu A, Brown KH, Peerson JM, Dewey KG. Predictors of growth from 1 to 18 months among breast-fed Ghanaian infants. Europ J Clin Nutr 54: 41-49, 2000.

Brown KH, Lutter CK. Potential role of processed complementary foods in the improvement of early childhood nutrition in Latin American. Food Nutr Bull 21: 5-11, 2000.

Cohen RJ, Brown KH, Rivera LL, Dewey KG. Exclusively breastfed, low birthweight term infants do not need supplemental water. Acta Paediatr 89: 550-552.

Brown KH. WHO/UNICEF review on complementary feeding and suggestions for future research: WHO/UNICEF guidelines on complementary feeding. Pediatrics 106:1290-1291, 2000

Zinc Investigators' Collaborative Group (Bhutta ZA, Bird SM, Black RE, Brown KH, Gardner JM, Hidayat A, Khatun F, Martorell R, Ninh NX, Penny ME, Rosado JL, Roy SK, Ruel M, Sazawal S, Shankar A). Therapeutic effects of oral zinc in acute and persistent diarrhea in children in developing countries: pooled analysis of randomized controlled trials. Am J Clin Nutr 72: 1516-1522, 2000.

Brown KH, Wuehler SE (eds.). Zinc and human health: results of recent trials and implications for program interventions and research. The Micronutrient Initiative, Ottawa, Canada, 2000.

Brown KH. Relations between gastrointestinal infections and childhood malnutrition. In: Suskind RM, Tontisirin K, eds., Nutrition, Immunity and Infection in Infants and Children. Philadelphia, PA: Lippincott, Williams & Wilkins, pp. 319-335, 2001

Mendoza C, Viteri FE, Lonnerdal B, Raboy V, Young KA, Brown KH. Absorption of iron from unmodified maize and genetically altered, low-phytate maize fortified with ferrous sulfate or sodium iron EDTA. Am J Clin Nutr 73: 80-85, 2001

Dewey KG, Cohen RJ, Brown KH, Rivera LL. Effects of exclusive breastfeeding for four versus six months on maternal nutritional status and infant motor development: Results of two randomized trials in Honduras. J Nutr 131: 262-267, 2001

Hotz C, Brown KH. Identifying populations at risk of zinc deficiency: the use of supplementation trials. Nut Rev 59: 80-84, 2001

Brown KH, Wuehler SE, Peerson JM. The importance of zinc in human nutrition and estimation of the global prevalence of zinc deficiency. Food Nutr Bull 22: 113-125, 2001

Rivera JA, Gonzalez-Cossio T, Flores M, Romero M, Rivera M, Tellez-Rojo MM, Rosado JL, Brown KH. Multiple micronutrient supplementation increases the growth of Mexican infants. Am J Clin Nutr 74: 657-663, 2001

Brown KH. A rational approach to feeding infants and young children with acute diarrhea. In: Lifschitz CH, ed., Pediatric gastroenterology and nutrition in clinical practice. New York: Marcel Dekker, Inc. 2001

Detailed Budget for New Proposal

Title: Estimation of the Average Vitamin A Requirement of AdultMales
PI: Dr. K.M.A. Jamil and Marjorie Haskell
Protocol Number: 2002-032 Name of Division: Clinical Sciences Division
Donor: USDA Amount Funded (direct): US$107,621 Total:US$128,069 Overhead:19%
Starting Date: January 2003 Closing Date: December 2005
Strategic Plan Priority Code(s):

Personnel	% Effort	1st Year	2nd Year	3rd Year	Total
Dr. Kazi M. Jamil, PI	50,50,20	5,600	5,880	2,470	13,950
Medical Officer, NA-1	50,50	3,888	4,083		7,971
Lab Supervisor (MA Waived), Co-investigator	5,5	900	945		1,845
Research Officer	100,50	4,128	2,168		6,296
Two Dieticians(Anowara,Hosne Ara)	10,10	1,200	1,260		2,460
Two Cooks, GS-1	100, 100	3,888	4,083		7,971
Field Supervisors, GS-2	100, 100	2,196	2,306		4,502
Two Field Assistant, GS-1	100, 100	1,944	2,042		3,986
Sub Total:		**23,744**	**22,767**	**2,470**	**48,981**
Local Travel(subject recruitment)		500	500		1,000
Sub Total:		**500**	**500**		**1,000**
Materials & Supplies:					
Medicines (Rx for parasites and for minor ailments)		250	250		500
Kitchen supplies		250			250
Food(2 major meals daily and snacks X 125 d)		12,000	12,000		24,000
Subject incentive (wage loss)		12,000	12,000		24,000
Subject incentive for screening		375	375		750
Microwave oven		250			250
Office supplies (stationeries)		150	150		300
Entertainment supplies (newspapers, journals, games materials)		250	250		500
Sub Total:		**25,525**	**25,025**		**50,550**
Other Contractual Services					
Rental of two airconditioners and other utilities		500	500		1,000
E-mail subscription, $30/m x 12 mo)		360	360		720
Sub Total:		**860**	**860**		**1,720**
Interdepartmental Services					
Microbiological tests		100	100		200
Biochemistry Test 150 screening; retinol, Hct, CRP, albumin, creatinine, SGPT, etc.		900	900		1,800
Xerox, fax, postal communications		200	250		450
Sub Total:		1,200	1,250		2,450

	1st Year	2nd Year	3rd Year	Total
Capital Expenditure				
Computer, printer and accessories	2,920			2,920
Sub Total:	2,920			2,920
Total Direct Cost:				107,621
Indirect costs(19%)				20,448
Total Project Costs				128,069

7/10/02

Md. Sazlur Rahman
Manager
Health &
Bangladesh

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: Dr. Jamil is the local PI who will be responsible for recruitment of subjects and for providing overall supervision of the project at ICDDR,B. The medical officer will collect blood samples from the subjects on specified time periods as mentioned in the protocol. He/she will also examine participants for signs and/or symptoms of vitamin A deficiency weekly throughout the study period.
The research officer will process all blood samples and store plasma samples at –20° C until the plasma samples are transported on dry ice to UC Davis. He/she will also share the responsibility of administering vitamin A capsules to the subjects together with the medical officer. The dietitians 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. The field supervisor will keep record of the attendance of the subjects and provide necessary support to look after the subjects during their stay at the study facility. He will also keep record of the daily intake of the subjects. The field assistants will help the field supervisor. The cooks and cleaners are self-explanatory.

Supplies: The computer will be used for data entry and analysis. The kitchen supplies and the microwave oven will be used for cooking food for the subjects. Newspapers, journals and game materials will be used for the recreation of the subjects who will spend all day in the study cafeteria for a period of 120 days consecutively.

Other Support

Describe sources, amount, duration, and grant number of all other research funding currently granted to PI or under consideration. (DO NOT EXCEED ONE PAGE FOR EACH INVESTIGATOR)

Dr. Jamil is currently receiving funds from NIH-Fogary International Center for conducting a pilot study on the antioxidant status of malnourished and healthy Bangladeshi children. The project started in March 1, 2002 and is expected to be completed in February 2003. The total budget for the pilot study was USS 3,000 (three thousand) only.

SCREENING

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

ICDDR,B: Centre for Health and Population Research & University of California Davis

Title of Study: Estimation of vitamin A requirements in adult males.

Investigators' Name(s), Department, Telephone Number(s):
K.M.A. Jamil, MBBS, Ph.D., CSD, ICDDR,B, Tel.(880-2) 8811751 Ext 2333
Marjorie Haskell, PhD, Dept of Nutrition, UC Davis, Tel. 1(530) 754-7415
Kenneth Brown, MD, Dept of Nutrition, UC Davis, Tel. 1(530) 752-1992

PURPOSE

You are being asked to participate in a screening procedure to determine whether you are eligible to participate in a research study. Researchers at the Centre for Health and Population Research in Bangladesh and the University of California-Davis are planning to conduct a research study to learn about how much vitamin A a person needs to consume in their daily diet to meet the body's vitamin A needs. We are conducting this screening procedure to identify volunteers with blood levels of vitamin A within the range of levels required for the research study.

PROCEDURES

If you decide to participate in this screening procedure to determine whether you are eligible for the study on vitamin A requirements, you will be asked to report the Centre for Health and Population Research at 7:00 am after a 12-hour overnight fast to have a blood sample obtained from a vein in your arm. The overnight fast means that you will be asked not eat or drink anything other than water after 7:00pm on the night before coming to the Centre. The blood sample will be obtained by inserting a needle in the vein in your arm and 2 teaspoons of blood (10 mL) will be removed for measurement of your blood vitamin A level. A physician will also ask you a few questions about your current state of health and your medical history. After the blood sample is obtained you will receive a light breakfast consisting of tea, a vegetable pastry, and a piece of fruit. We will let you know within 2 weeks whether or not you are eligible for the research study on vitamin A requirements. If you are eligible for the study and are interested in participating, we will explain the study procedures to you in detail at that time. Your participation in this screening procedure does not obligate you in any way to participate in the research study on vitamin A requirements.

ALTERNATIVES

The alternative is not to participate in the study.

RISKS

There are no major risks associated with your participation in this screening procedure. Risks of removing blood from a vein in your arm include some discomfort, bruising, dizziness

Participant's initial _____

and rarely infection. The amount of blood that will be taken will not affect your health in any way.

BENEFITS

No direct benefit to you is anticipated from participating in this screening procedure. Results of the study on vitamin A requirements will benefit society in general because, at present, there is very little information on the amount of vitamin A that is needed to meet the body's needs. This information will be useful to public health professionals for establishing programs and policies to address the problem of vitamin A deficiency worldwide.

CONFIDENTIALITY

The information obtained from this screening procedure will be shared between the Center for Health and Population Research in Bangladesh and the University of California, Davis. Names and personal information will be kept confidential to protect your identity, however, absolute confidentiality cannot be guaranteed, since research documents are not protected from subpoena.

COSTS/COMPENSATION

You will be asked to provide us with your time. There will be no cost to you to participate in this study. **You will receive 170 Taka as compensation for the time you will spend at the study facility and for reimbursement of your transportation costs to and from the Center for Health and Population Research**. If you are injured as a direct result of research procedures, you will receive reasonably necessary medical treatment at no cost. The Center for Health and Population Research in Bangladesh and the University of California-Davis do not provide any other form of compensation for injury.

RIGHT TO REFUSE OR WITHDRAW

Participation in this screening procedure is voluntary. If you decide to participate, you are free to 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, Dr. Jamil, Dr. Haskell, Dr. Brown or one of their assistants will answer them. Dr. Jamil can be reached at the Centre for Population Studies, Mohakhali, Dhaka, Bangladesh, Tel. 8811751 Ext 2333. Dr. Haskell and Dr. Brown can be reached at 3217 Meyer Hall, UC Davis, Davis, CA 95616, Tel. 001-530-752-1992.

CONSENT

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

Participant's initial _____

Name of participant (print): _____

Signature or thumbprint of participant : _____ **Date**_____

Name of investigator (print): _____

Signature of investigator:_____ **Date**_____

Name of witness (print): _____

Signature of witness: _____ **Date**_____

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.

Participant's initial _____

CONSENT FORM 1

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

ICDDR,B: Centre for Health and Population Research & University of California Davis

Title of Study: Estimation of vitamin A requirements in adult males.

Investigators' Name(s), Department, Telephone Number(s):
K.M.A. Jamil, MBBS, Ph.D., CSD, ICDDR,B, Tel.(880-2) 8811751 Ext 2333
Marjorie Haskell, PhD, Dept of Nutrition, UC Davis, Tel. 1(530) 754-7415
Kenneth Brown, MD, Dept of Nutrition, UC Davis, Tel. 1(530) 752-1992

PURPOSE

You are being asked to participate in a research study. Researchers at the Centre for Health and Population Research in Bangladesh and the University of California-Davis hope to learn how much vitamin A a person needs to consume in their daily diet to meet the body's vitamin A needs.

PROCEDURES

If you decide to volunteer for the study, you will be asked to consume all of your meals during the next four months in the study cafeteria at the Centre for Health and Population Research. You will be asked to report to the study facility at 7:00 am and remain there under supervision until 7:00 pm. During that time you will be provided with breakfast, lunch, dinner and snacks. Games and newspapers will be made available at the study facility to alleviate boredom. You will be allowed to exercise outside, and space will be made available for resting. In addition to the meals, you will also receive a vitamin and mineral supplement daily. The meals and the vitamin and mineral supplements that you will be given will satisfy all of your nutrient requirements except for vitamin A. You will be asked not to eat or drink anything other than water when you return to your home at night. After three weeks of consuming your meals in the study cafeteria, you will begin receiving a capsule (with your noontime and evening meals) containing 100, 200, 300, 400, 500, 600, 800, or 1000 μg of vitamin A twice daily for a period of 2 months. The amount of vitamin A that you will receive in the capsules will be determined by chance, as in a lottery. During those 2 months you will continue to receive the meals and capsules at the study cafeteria. After the 2 month period, you will continue to consume your meals in the study cafeteria for approximately 5 more weeks, but during that time you will no longer receive the daily vitamin A capsules.

Additional procedures that will be completed are described as follows:

1. On the third day of the study, you will receive a small dose of "heavy vitamin A" (10 mg) by mouth, which will allow us to estimate how much vitamin A is present in your body. "Heavy vitamin A" is a non-toxic form of vitamin A that is slightly heavier than the vitamin A that is found in foods. **We will obtain a blood sample from a vein in your arm.** A needle will be inserted in the vein and 2 teaspoons of blood (10 mL) will be removed 24 hours after you receive the dose of "heavy vitamin A", and again 3, 20 and 21 days after receiving the dose. On

Participant's initial _____

the following day you will begin receiving a capsule containing vitamin A, as described above, with the noon and evening meals for a period of 2 months.

2. Following the two-month period, we will obtain a blood sample from a vein in your arm to measure blood levels of vitamin A. A needle will be inserted in the vein and 2 teaspoons of blood (10 mL) will be removed. You will continue to consume your meals in the study cafeteria for approximately 5 more weeks. During those 5 weeks you will no longer receive daily vitamin A capsules. You will receive the final dose of "heavy vitamin A" (10 mg) by mouth, two weeks after completing the 2-month supplementation period. The "heavy vitamin A" 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 absorbed from the capsules that you received during the two-month period. To complete this study, we will obtain a blood sample from a vein in your arm. A needle will be inserted in the vein and 2 teaspoons of blood (10 mL) will be removed on the morning that you receive the dose of "heavy vitamin A", and again 1, 3, 20 and 21 days after you receive the dose.

3. The whole study will require 120 days (4 months). A total of 10 blood samples (2 teaspoons each (10 mL each)) will be obtained over the 120-day period.

ALTERNATIVES

The alternative is not to participate in the study.

RISKS

There are no major risks associated with your participation in this study. Risks of removing blood from a vein in your arm include some discomfort, bruising, dizziness 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 that poses no risk to you. The amount of vitamin A that you will be receiving daily during the supplementation period is below the Upper Limit (3000 µg/d) established by the Institute of Medicine; therefore you will not be at risk of consuming too much vitamin A daily. There is a chance that you will be receiving as little as 100 µg/d of vitamin A, which is less than the current recommended daily allowance of 700 µg/d for adult males, or as much as 1000 µg/d of vitamin A during the supplementation period. There is a very slight risk that you may develop symptoms of vitamin A deficiency if you are consuming less than the recommended daily allowance for vitamin A during the study. However, the basal low-vitamin A study diet contains approximately 75-100 µg of vitamin A, thus subjects who receive a daily dose of 100 µg as vitamin A capsules will receive a total of 175-200 µg/d from the diet and capsules combined. It is very unlikely that clinical signs of vitamin A deficiency will occur at this level of intake. The study physician will examine you weekly, and if any symptoms of vitamin A deficiency occur you will be treated with vitamin A immediately. Early clinical symptoms of vitamin A deficiency include difficulty seeing in dim light, and dryness of the white part of the eye. However, in previous studies in which Bangladeshi men received 0 mg vitamin A/d for a period of 120 days, clinical symptoms of vitamin A deficiency did not occur in any of the subjects. Upon completion of the study, you will be given a single, high-dose vitamin A supplement (60 mg retinyl palmitate) to replete your vitamin A stores.

Participant's initial _____

BENEFITS

Results of this study will benefit society in general because, at present, there is very little information on the amount of vitamin A that is needed to meet the body's needs. This information will be useful to public health professionals for establishing programs and policies to address the problem of vitamin A deficiency worldwide. No direct benefit to you is anticipated.

CONFIDENTIALITY

The information obtained from this study will be shared between the Center for Health and Population Research in Bangladesh and the University of California, Davis. Names and personal information will be kept confidential to protect your identity, however, absolute confidentiality cannot be guaranteed, since research documents are not protected from subpoena.

COSTS/COMPENSATION

You will be asked to provide us with your time. There will be no cost to you to participate in this study. You will receive monetary compensation for the time you spend at the study facility (7:00am-7:00pm daily). Specifically, you will receive **170 Taka/d** for 120 days. The compensation will be provided to you **every 10 days (1,700 Taka/10 days); If you decide to withdraw before completing the study, you will be paid for the number of days that you participated in the study at a rate of 170 Taka/d.** If you are injured as a direct result of research procedures, you will receive reasonably necessary medical treatment at no cost. The Center for Health and Population Research in Bangladesh and the University of California-Davis do not provide any other form of compensation for injury.

RIGHT TO REFUSE OR WITHDRAW

Participation in this study is voluntary. If you decide to participate, you are free to 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, Dr. Jamil, Dr. Haskell, Dr. Brown or one of their assistants will answer them. Dr. Jamil can be reached at the Centre for Population Studies, Mohakhali, Dhaka, Bangladesh, Tel. 8811751 Ext 2333. Dr. Haskell and Dr. Brown can be reached at 3217 Meyer Hall, UC Davis, Davis, CA 95616, Tel. 001-530-752-1992.

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

Participant's initial _____

Name of participant (print): _____

Signature or thumbprint of participant : _____ **Date**_____

Name of investigator (print): _____

Signature of investigator:_____ **Date**_____

Name of witness (print): _____

Signature of witness: _____ **Date**_____

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.

Participant's initial _____

mouth, which will allow us to estimate how much vitamin A is present in your body. **We will obtain a blood sample from a vein in your arm**. A needle will be inserted in the vein and 2 teaspoons (10 mL) of blood will be removed 24 hours after you receive the dose of "heavy vitamin A", and again 3, 20 and 21 days after receiving the dose. On the following day you will begin receiving a capsule containing vitamin A, as described above, with the noon and evening meals for a period of 2 months.

3. Following the two-month period, we will obtain a blood sample from a vein in your arm to measure blood levels of vitamin A. A needle will be inserted in the vein and 2 teaspoons (10 mL) of blood will be removed. You will continue to consume your meals in the study cafeteria for approximately 5 more weeks. During that time you will no longer receive daily capsules of vitamin A. You will receive the final dose of "heavy vitamin A" (15 mg) by mouth two weeks after completing the 2-month supplementation period. The "heavy vitamin A" 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 absorbed from the capsules that you received during the two-month period. To complete this study, we will obtain a blood sample from a vein in your arm. A needle will be inserted in the vein and 2 teaspoons (10 mL) of blood will be removed on the morning that you receive the dose of "heavy vitamin A", and again 1, 3, 20 and 21 days after you receive the dose.

PROCEDURES

4. The whole study period will require 120 days (4 months). A total of 10 blood samples will be obtained (two teaspoons each (10 mL each)) over the 120 day study period.

ALTERNATIVES

The alternative is not to participate in the study.

RISKS

There are no major risks associated with your participation in this study. Risks of removing blood from a vein in your arm include some discomfort, bruising, dizziness 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. The amount of vitamin A that you will be receiving daily during the supplementation period is below the Upper Limit (3000 µg/d) established by the Institute of Medicine. There is a chance that you will be receiving as little as 275-300 µg/d of vitamin A (from the study diet and capsules combined) which is less than the current recommended daily allowance of 700 µg/d for adult males. However, in previous studies in which Bangladeshi men received 0 mg vitamin A/d for a period of 120 days, clinical symptoms of vitamin A deficiency did not occur in any of the subjects. Also, it is very unlikely that you will develop symptoms of vitamin A deficiency if you are consuming less than 700 µg/d during the supplementation period because prior to beginning the studies you will have received 4 doses of 60 mg of vitamin A. This amount of vitamin A will increase the amount of vitamin A in your body to a higher level and should be sufficient to protect against risk of vitamin A deficiency for a period of approximately 4 months even if no vitamin A were consumed in your diet. Nevertheless, the study physician will examine you

Participant's initial _____

weekly, and if any symptoms of vitamin A deficiency occur you will be treated with vitamin A immediately. Early clinical symptoms of vitamin A deficiency include difficulty seeing in dim light, and dryness of the white part of the eye. It is possible that you may experience some side effects during the first day after taking a high dose capsule (60 mg) such as headache, nausea or vomiting. However, these side effects generally occur in a small number of people who receive high-dose supplements (less than 5%) and do not result in any long-term health problems. As described, you will receive four high-dose capsules 5 days apart before beginning the 4-month study.

BENEFITS

Results of this study will benefit society in general because, at present, there is very little information on the amount of vitamin A that is needed to meet the body's needs. This information will be useful to public health professionals for establishing programs and policies to address the problem of vitamin A deficiency worldwide. No direct benefit to you is anticipated.

CONFIDENTIALITY

The information obtained from this study will be shared between the Center for Health and Population Research in Bangladesh and the University of California, Davis. Names and personal information will be kept confidential to protect your identity, however, absolute confidentiality cannot be guaranteed, since research documents are not protected from subpoena.

COSTS/COMPENSATION

You will be asked to provide us with your time. There will be no cost to you to participate in this study. You will receive monetary compensation for the time that you spend at the study facility (7:00am-7:00pm daily). **Specifically, you will receive 170 Taka/d for 120 days. The compensation will be provided to you every 10 days (1,700 Taka/10 days). If you decide to withdraw before completing the study, you will be paid for the number of days that you participated in the study at a rate of 170 Taka/d.** If you are injured as a direct result of research procedures, you will receive reasonably necessary medical treatment at no cost. The Center for Health and Population Research in Bangladesh and the University of California-Davis do not provide any other form of compensation for injury.

RIGHT TO REFUSE OR WITHDRAW

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QUESTIONS

If you have any questions, please ask us. If you have additional questions later, Dr. Jamil, Dr. Haskell, Dr. Brown or one of their assistants will answer them. Dr. Jamil can be reached at the Centre for Population Studies, Mohakhali, Dhaka, Bangladesh, Tel. 8811751 Ext 2333. Dr. Haskell and Dr. Brown can be reached at 3217 Meyer Hall, UC Davis, Davis, CA 95616, Tel. 001-530-752-1992.

Participant's initial _____

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Name of participant (print): _____

Signature or thumbprint of participant : _____ Date_____

Name of investigator (print): _____

Signature of investigator:_____ Date_____

Name of witness (print): _____

Signature of witness: _____ Date_____

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.

Participant's initial _____

(বাছাই পর্ব)

"একটি গবেষনায় অংশগ্রহন করার জন্য সম্মতি পত্র"

ইউনির্ভাসিটি অফ ক্যালিফোর্নিয়া ডেভিস ও আই.সি.ডি.ডি.আর,বি : সেন্টার ফর হেল্‌থ এন্ড পপুলেশন রির্সাচ

গবেষনার নাম ঃ প্রাপ্তবয়স্ক পুরুষের জন্য ভিটামিন 'এ'-র প্রয়োজনীয়তা নির্ণয় - রক্তের রেটিনল মাপার জন্য ব্যাছাই পর্ব'

গবেষকদের নাম, বিভাগ, ফোন নং ঃ
ডাঃ কাজী মোঃ আসিফ জামিল, সি.এস.ডি, আই.সি.ডি.ডি.আর,বি, ফোন- (৮৮০-২) ৮৮১১৭৫১ এক্স ২৩৩৩
ডঃ মার্জোরি হাস্কেল, পুষ্টিবিদ্যা, ইউ,সি,ডেভিস ১ (৫৩০-৭৫৪-৭৪১৫);
ডাঃ কেনেথ ব্রাউন, পুষ্টিবিদ্যা, ইউ,সি,ডেভিস ১ (৫৩০-৭৫২-১৯৯২)

উদ্দেশ্য ঃ
আপনাকে একটি গবেষনার বাছাই পর্বে যোগ দেয়ার জন্য আহ্বান করা হচ্ছে যার মাধ্যমে ঐ গবেষনায় আপনার অংশ গ্রহন করার যোগ্যতা নির্ণয় করা হবে। বাংলাদেশের আই,সি,ডি,ডি, আর,বি ও ইউনিভার্সিটি অফ ক্যালির্ফোনিয়া ডেভিসের গবেষকরা একজন ব্যক্তির দৈনিক চাহিদা মিটানোর জন্য তার খাদ্যে কতটুকু পরিমান ভিটামিন 'এ' থাকা উচিত তা নির্ণয় করার জন্য এ গবেষনা চালাবেন। স্বেচ্ছায় অংশগ্রহনকারীদের রক্তে ভিটামিন 'এ' -র মাত্রা এই গবেষনার জন্য প্রয়োজনীয় সীমার মধ্যে রয়েছে কিনা তা এই বাছাই পর্বে পরীক্ষা করা হবে।

পদ্ধতি ঃ
মানবদেহে ভিটামিন 'এ'-র প্রয়োজনীয়তা নির্নয়ের উদ্দেশ্যে আয়োজিত এই গবেষনার 'বাছাই পর্বে' আপনি যদি অংশ সিতে চান তাহলে আপনাকে ১২ ঘন্টা উপবাসের পর সকাল ৭টার সময় হাতের শিরা থেকে রক্তদানের জন্য আই,সি,ডি,ডি, আর, বিতে আসতে বলা হবে। অর্থাৎ আপনি যেদিন রক্তদান করবেন তার পূর্ববর্তী রাত ৭টা থেকে পরদিন সকাল ৭টা পযর্ন্ত শুধুমাত্র পানি পান করা ছাড়া অন্যান্য সকল পানাহার থেকে আপনাকে বিরত থাকতে হবে। রক্ত সংগ্রহের জন্য আপনার হাতের শিরায় সুঁই ঢুকিয়ে প্রায় দু চা চামচ (১০ মিঃ লিঃ) পরিমান রক্ত নেয়া হবে এব তাতে ভিটামিন 'এ'-র মাত্রা নির্ণয় করা হবে। এছাড়া একজন ডাক্তার আপনাকে আপনর স্বাস্থ্য বিষয়ক কিছু প্রশ্ন করবেন। রক্ত সংগ্রহের পর আপনাকে সকালের নাস্তা পরিবেশন করা হবে যার মধ্যে চা, সবজির পেস্ট্রি ও ক টুরা ফল দেয়া হব। আপনি ভিটামিন 'এ' প্রয়োজন নির্নয়মূলক গবেষনায় অংশগ্রহনের জন্য উপযুক্ত কি না তা আপনাকে পরবর্তী দুই সপ্তাহের মধ্যে জানিয়ে দেয়া হবে। যদি আপনি এই গবেষনায় অংশগ্রহনের জন্য যোগ্য বিবেচিত হন এবং এতে যোগ দিতে আগ্রহী হন তাহলে ঐ সময় আপনাকে গবেষনার পদ্ধতি সম্পর্কে বিস্তারিত ব্যাখ্যা দেয়া হবে। আপনি গবেষনার বাছাই পর্বে অংশগ্রহন করলেও পরবর্তী পর্যায়ে মূল গবেষনায় যোগ দেয়ার জন্য কোন বাধ্যবাধকতা থাকবে না।

বিকল্পঃ
বিকল্প উপায় হচ্ছে গবেষণায় অংশগ্রহণ না করা।

ঝুঁকিসমূহ ঃ
এই গবেষনার বাছাই পর্বে অংশগ্রহনের ফলে আপনাকে বিশেষ কোন ঝুঁকির সম্মুখীন হতে হবে না। শিরা থেকে রক্ত নেয়ার সময় সামান্য ব্যথা, ক্ষতসৃষ্টি, মাথা ঘুরানো বা কখনো কখনো জীবানুর সংক্রমন হবার ক্ষীন সম্ভাবনা থাকে। যেটুকু রক্ত আপনার শরীর থেকে নেয়া হবে তাতে আপনার স্বাস্থ্যের কোন ক্ষতি হবে না।

উপকারিতা ঃ
এই গবেষনার বাছাই পর্বে অংশগ্রহনের মাধ্যমে আপনি সরাসরি উপকৃত হবার কোন সম্ভাবনা নেই। তবে ভিটামিন 'এ'-র প্রয়োজনীয়তা সম্পর্কে গবেষনার ফলাফল সামগ্রিকভাবে সমাজের সবার উপকারে আসবে। কারণ শরীরের চাহিদা মিটানোর জন্য ঠিক কি পরিমান ভিটামিন 'এ-র প্রয়োজন সে সম্পর্কে আমাদের ধারনা অপ্রতুল। জনস্বাস্থ্য বিশেষজ্ঞরা এই গবেষনালব্ধ তথ্যকে কাজে লাগিয়ে বিশ্বের বিভিন্ন স্থানে ভিটামিন 'এ'-র অভাবদূর করার জন্য প্রয়োজনীয় পদক্ষেপ নিতে পারবেন।

গোপনীয়তা ঃ

গবেষনার বাছাই পর্বে প্রাপ্ত তথ্য শুধুমাত্র আই, সি, ডি,ডি, আর,বি ও ইউনিভার্সিটি অফ ক্যালিফোর্নিয়া ডেভিসে রক্ষিত থাকবে। কোন আইনগত কারনে প্রয়োজন না হলে আপনার নাম ও অন্যান্য ব্যক্তিগত তথ্যসমূহ সম্পূর্ণ গোপন রাখা হবে।

মূল্য/ক্ষতিপূরন ঃ

এই গবেষনার জন্য আমরা আপনাকে কিছু সময় দিতে অনুরোধ করবো। গবেষনায় অংশগ্রহনের জন্য আপনাকে কোন খরচ বহন করতে হবে না। আমাদেরকে গবেষনা ক্ষেত্রে আপনার সময় (সকাল ৭:০০ - ৮:০০টা) দেয়ার জন্য আপনাকে আর্থিক ক্ষতিপূরন দেয়া হবে। যদি গবেষনা প্রক্রিয়ায় আপনার কোন শারীরিক ক্ষতি হয় তাহলে আপনাকে বিনামূল্যে চিকিৎসা করা হবে। আই,সি,ডি,ডি, আর, বি বা ইউনিভার্সিটি অফ ক্যালিফোর্নিয়া ডেভিস এছাড়া অন্য কোন ধরনের ক্ষতিপূরন দিতে অপারগ।

গবেষনায় অংশগ্রহন প্রত্যাহার করার অধিকার ঃ

গবেষনার বাছাই পর্বে স্বতঃস্ফুর্তভাবে অংশগ্রহন করার জন্য আপনাকে আহবান করা হচ্ছে। আপনি গবেষনায় অংশগ্রহনে সম্মত হলেও বাছাইয়ের পরে যেকোন সময় আপনার নাম প্রত্যাহার করতে পারেন।

প্রশ্নোত্তর ঃ

আপনার কোন প্রশ্ন থাকলে অনুগ্রহ করে জিজ্ঞাসা করুন। আপনার মনে যদি পরেও কোন প্রশ্ন জাগে সেক্ষেত্রে আপনার প্রশ্নের উত্তর ডাঃ জামিল, ডঃ হাস্কেল, ডাঃ ব্রাউন বা তাদের অধ্যস্তন কর্মীরা দিবেন। ডাঃ জামিলের ঠিকানা আই,সি,ডি,ডি,আর, বি মহাখালী, ঢাকা। ফোন নং - ৮৮১১৭৫১/২৩৩৩। ডঃ হাস্কেল ও ডাঃ ব্রাউনের ঠিকানা ঃ 3217 MEYER HALL, UC DAVIS, DAVIS, CA 95616, Tel. 001-530-752-1992.

সম্মতি ঃ

আপনার নীচের স্বাক্ষরটি এই অর্থ বহন করবে যে আপনি স্বেচ্ছায় গবেষনায় অংশগ্রহন করছেন এবং আপনি উপরে বর্নিত তথ্যসমুহ এবং আপনার অধিকার সম্পর্কে পড়েছে এবং বুঝতে পেরেছেন।

অংশগ্রহণকারীর নাম : _____

অংশগ্রহণকারীর স্বাক্ষর : _____ তারিখ : _____

গবেষকের নাম : _____

গবেষকের স্বাক্ষর : _____ তারিখ : _____

সাক্ষীর নাম : _____

সাক্ষীর স্বাক্ষর : _____ তারিখ : _____

আপনাকে স্বাক্ষর ও তারিখ সহকারে এই দলিলের একটি কপি সংরক্ষন করার জন্য দেয়া হবে। এ ছাড়া আপনাকে গবেষণা কাজে অংশগ্রহনকারী ব্যক্তির অধিকার সম্পর্কিত তথ্যও দেয়া হবে।

অংশগ্রহণকারীর সহি _____

(সম্মতি পত্র ১)

"একটি গবেষনায় অংশগ্রহন করার জন্য সম্মতি পত্র"

ইউনির্ভাসিটি অফ ক্যালিফোর্নিয়া ডেভিস ও আই.সি.ডি.ডি.আর,বি : সেন্টার ফর হেল্‌থ এন্ড পপুলেশন রির্সাচ

গবেষনার নাম ঃ প্রাপ্তবয়স্ক পুরুষের জন্য ভিটামিন 'এ'-র প্রয়োজনীয়তা নির্ণয়

গবেষকদের নাম, বিভাগ, ফোন নং ঃ
ডাঃ কাজী মোঃ আসিফ জামিল, সি.এস.ডি, আই.সি.ডি.ডি.আর,বি, ফোন- (৮৮০-২) ৮৮১১৭৫১ এক্স ২৩৩৩
ডঃ মার্জোরি হাস্কেল, পুষ্টিবিদ্যা, ইউ,সি,ডেভিস (০০১) ৫৩০-৭৫৪-৭৪১৫;
ডাঃ কেনেথ ব্রাউন, পুষ্টিবিদ্যা, ইউ,সি,ডেভিস (০০১) ৫৩০-৭৫২-১৯৯২

উদ্দেশ্য ঃ
আপনাকে একটি গবেষনায় অংশগ্রহন করার জন্য আহ্বান করা হচ্ছে। বাংলাদেশের আই,সি,ডি,ডি, আর,বি ও ইউনিভার্সিটি অফ ক্যালির্ফোনিয়া ডেভিসের গবেষকরা একজন ব্যক্তির দৈনিক চাহিদা মিটানোর জন্য তার খাদ্যে কতটুকু পরিমান ভিটামিন 'এ' থাকা উচিত তা নির্ণয় করার জন্য এ গবেষনা চালাবেন।

পদ্ধতি ঃ
আপনি যদি স্বেচ্ছায় এই গবেষনায় যোগ দিতে চান তাহলে আপনাকে আগামী চার মাস আই,সি,ডি,ডি, আর, বি ক্যাফেটেরিয়াতে যে খাবার পরিবেশন করা হবে তার সবটুকু খেতে বলা হবে। আপনাকে প্রতিদিন সকাল ৭টার সময় গবেষনা ক্ষেত্রে উপস্থিত হতে হবে এবং সন্ধা ৭টা পর্যন্ত সেখানে আমাদের তত্ত্বাবধানে থাকতে হবে। এসময় আপনাকে সকালের নাস্তা, দুপুর ও রাতের খাবার এবং অন্য সময়ে হালকা নাস্তা পরিবেশন করা হবে। এখানে আপনার চিত্ত বিনোদনের জন্য খেলাধূলা ও সংবাদ পত্র পড়ার ব্যবস্থা থাকবে। আপনার জন্য বাইরে ব্যায়াম করার ব্যবস্থা ও গবেষনা ক্ষেত্রে বিশ্রাম নেয়ার ব্যবস্থা থাকবে।

খাবার সরবরাহের পাশাপাশি আপনাকে প্রতিদিন ভিটামিন ও খনিজ লবন পরিবেশন করা হবে। আপনাকে প্রতিদিন যেসব খাবার, ভিটামিন ও খনিজ লবন সরবরাহ করা হবে তাতে আপনার শরীরের ভিটামিন 'এ' ছাড়া অনান্য সকল পুষ্টির চাহিদা পূরন হবে। আপনাকে রাত্রে বাসায় ফিরার পর শুধুমাত্র পানি ছাড়া অন্যান্য সকল খাবার ও পানীয় গ্রহন করা থেকে বিরত থাকার জন্য অনুরোধ জানানো হবে। ক্যাফেটেরিয়াতে তিন সপ্তাহ খাওয়া দাওয়া করার পর আপনাকে পরবর্তী দুই মাস ধরে দুপুর ও রাতের খাবারের সঙ্গে প্রতিবেলায় একটি করে দিনে দুইটি ক্যাপসুল খেতে দেয়া হবে। এ ক্যাপসুলে বিভিন্ন মাত্রার, যেমন ১০০, ২০০, ৩০০, ৪০০, ৫০০, ৬০০, ৮০০ বা ১০০০ মাইক্রোগ্রাম পরিমান ভিটামিন 'এ' থাকবে। আপনাকে কোন মাত্রার ভিটামিন 'এ' দেয়া হবে তা লটারীর মাধ্যমে নির্ধারন করা হবে। এই দুইমাস ধরে আপনাকে ক্যাফেটেরিয়াতে যে খাবার পরিবেশন করা হবে তার সবটুকু খেতে বলা হবে। দুই মাস অতিক্রান্ত হবার পর আরও পাঁচ সপ্তাহ ধরে আপনাকে ক্যাফেটেরিয়াতে খেতে হবে - তবে এই সময়ে কোন ভিটামিন 'এ' ক্যাপসুল খেতে দেয়া হবে না।

বিকল্পঃ
বিকল্প উপায় হচ্ছে গবেষণায় অংশগ্রহণ না করা।

গবেষনার অন্যান্য প্রক্রিয়াগুলির বিবরন নীচে দেয়া হলো :

১। গবেষনার তৃতীয় দিনে আপনাকে স্বল্পমাত্রার (১০ মিঃ লিঃ গ্রাম) 'ভারী' ভিটামিন 'এ' খেতে দেয়া হবে। যাতে আপনার শরীরে কি পরিমান ভিটামিন 'এ' আছে তা নির্ণয় করা যায়। ভারী ভিটামিন 'এ'-র তেমন কোন ক্ষতিকর প্রভাব নেই - এটি খাদ্যের মধ্যে অবস্থিত ভিটামিন 'এ'-র তুলনায় কিছুটা ভারী। ভারী ভিটামিন 'এ' খাওয়ার ২৪ ঘন্টা পর আপনার হাতের শিরায় সুঁই ঢুকিয়ে দুই চা চামচ (১০ মিঃ লিঃ) পরিমান রক্ত সংগ্রহ করা হবে। অনুরুপভাবে, ভারী ভিটামিন 'এ' সেবনের ৩, ২০ ও ২১ দিন পরও আপনার হাতের শিরা থেকে প্রতিবার দুই চা চামচ ১০ মিঃ লিঃ) পরিমান রক্ত

নেয়া হবে। এর পরদিন থেকে পরবর্তী দুই মাস ধরে আপনাকে দুপুর ও রাতের খাবারের সঙ্গে ভিটামিন 'এ' ক্যাপসুল খেতে দেয়া হবে।

২। দুইমাস অতিক্রান্ত হবার পর রক্তে ভিটামিন 'এ'-র মাত্রা নির্ণয়ের জন্য আপনার হাতের শিরায় সুঁই ঢুকিয়ে দুই চা চামচ (১০ মিঃ লিঃ) পরিমান রক্ত নেয়া হবে। তারপর আরও পাঁচ সপ্তাহ ধরে আপনাকে ক্যাফেটেরিয়তে খেতে হবে। এই সময়ে আপনাকে কোন ভিটামিন 'এ' ক্যাপসুল খেতে দেয়া হবে না। ক্যাফেটেরিয়তে দুই মাস ভারী ভিটামিন 'এ' খাওয়ানো শেষ হবার আরও দুই সপ্তাহ পর শেষবারের মত ভারী ভিটামিন 'এ' (১০ মিঃ লিঃ) খেতে দেয়া হবে। আপনার শরীরে পুনরায় ভিটামিন 'এ' - পরিমান নির্ণয় করার জন্যই ভী ভিটামিন 'এ' খাওয়ানো হবে। দুই মাস ধরে ভিটামিন 'এ' ক্যাপসুল সেবনের পর আপনার শরীরে কতটুকু ভিটামিন 'এ' সংগৃহীত হলো তা এই পরীক্ষার মাধ্যমে জানা যাবে। পরীক্ষা সম্পন্ন করার জন্য ভারী ভিটামিন 'এ' গ্রহন করার দিন সকালে ও তার পরবর্তী ১, ৩, ২০ ও ২১ দিন পর আপনার শিরা থেকে প্রতিবার দুই চা চামচ (১০ মিঃ লিঃ) পরিমান রক্ত নেয়া হবে।

৩। গবেষনা শেষ হতে মোট ১২০ দিন (৪ মাস) সময় লাগবে। এই সময়ে মোট ১০ বার (প্রতিবার ২ চা চামচ বা ১০ মিঃ লিঃ পরিমান) রক্ত সংগ্রহ করা হবে।

ঝুঁকিসমূহ ঃ

এই গবেষনার অংশগ্রহনের ফলে আপনাকে বিশেষ কোন ঝুঁকির সম্মুখীন হতে হবে না। শিরা থেকে রক্ত নেবার সময় সামান্য ব্যথা, ক্ষতসৃষ্টি, মাথা ঘুরানো বা কখনো কখনো জীবানুর সংক্রমন হওয়ার ক্ষীন সম্ভাবনা থাকে। যেটুকু রক্ত আপনার শরীর থেকে নেয়া হবে তাতে আপনার স্বাস্থ্যের কোন ক্ষতি হবে না। বিশেষ ধরনের "ভারী ভিটামিন এ" কোন ক্ষতিকারক জিনিস নয় এবং এটা সেবনে কোন ঝুঁকি নেই। আপনাকে প্রতিদিন যে পরিমান ভিটামিন 'এ' খেতে দেয়া হবে তা 'চিকিৎসা বিজ্ঞান ইনস্টিটিউট'-এর মতে 'সর্বোচ্চ মাত্রা' (প্রতিদিন ৩০০০ মাইক্রোগ্রাম) থেকে অনেক কম। সুতরাং এই গবেষনার আপনার অতিরিক্ত ভিটামিন 'এ' সেবনের কোন ঝুঁকি নেই। আপনাকে হয়তো প্রতিদিন সর্বনিম্ন ১০০ মাইক্রো গ্রাম বা সর্বোচ্চ ১০০০ মাইক্রো গ্রাম ভিটামিন 'এ' খেতে দেয়া হবে। এখানে উল্লেখ্য যে একজন প্রাপ্তবয়স্ক পুরুষের জন্য ভিটামিন 'এ'-র দৈনিক নির্দেশিত বরাদ্দের (RDA) পরিমান হচ্ছে ৭০০ মাইক্রো গ্রাম। আপনাকে যদি এই গবেষনায় 'দৈনিক নির্দেশিত বরাদ্দের' (RDA) চাইতে কম পরিমান ভিটামিন 'এ' খেতে দেয়া হয়, তাতে আপনার শরীরে ভিটামিন 'এ' -র অভাবজনিত উপসর্গ দেখা দেওয়ার সম্ভাবনা অত্যন্ত ক্ষীন, গবেষনা চলাকালীন সময়ে আপনাকে স্বল্প ভিটামিন 'এ'-যুক্ত যে মূল খাবার (BASAL DIET) দেয়া হবে তাতে ৭৫-১০০ মাইক্রো গ্রাম ভিটামিন 'এ' থাকবে। অতএব, যারা প্রতিদিন ১০০ মাইক্রো গ্রাম মাত্রার ভিটামিন 'এ' ক্যাপসুল পাবে, প্রকৃত পক্ষে তারা খাবারে যুক্ত ভিটামিন 'এ' সহ দৈনিক মোট ১৭৫-২০০ মাইক্রো গ্রাম ভিটামিন 'এ' গ্রহন করবে। এই পরিমান ভিটামিন 'এ' গ্রহন করার পর ভিটামিন 'এ'-র অভাবজনিত উপসর্গ দেখা যাওয়ার সম্ভাবনা নেই বললেই চলে। গবেষনার দায়িত্বে নিয়োজিত চিকিৎসক প্রতি সপ্তাহে আপনার স্বাস্থ্য পরীক্ষা করবেন, এবং ভিটামিন 'এ'-র অভাবজনিত কোন সমস্যা দেখা গেলে সঙ্গে সঙ্গে ভিটামিন 'এ' দিয়ে চিকিৎসা করবেন। শরীরে ভিটামিন 'এ'-র অভাব হলে কম আলোয় দেখতে কষ্ট হওয়া, চোখের সাদা অংশ শুকিয়ে যাওয়া ইত্যাদি উপসর্গ প্রাথমিক পর্যায়ে দেখা যেতে পারে। তবে ইতিপূর্বে বাংলাদেশে গবেষনায় দেখা গেছে, ১২০ দিন পর্যন্ত প্রতিদিন সম্পূর্ন ভিটামিন 'এ' - বিহীন খাবার সরবরাহ করা সত্ত্বেও ঐসব ব্যক্তির শরীরে ভিটামিন 'এ'-র অভাবজনিত কোন উপসর্গ দেখা যায়নি। গবেষনার শেষে আপনাকে একটি উচ্চ মাত্রার ভিটামিন 'এ' (৬০ মিঃ গ্রাঃ রেটিনল পালমিটেট) ক্যাপসুল খেতে দেয়া হবে যাতে আপনার শরীরে ভিটামিন 'এ'-র কোন ঘাটতি না থাকে।

উপকারিতা ঃ

আমাদের এই গবেষনার ফলাফল সামগ্রিকভাবে সমাজের সবার উপকারে আসবে। কারন শরীরের চাহিদা মিটানোর জন্য ঠিক কি পরিমান ভিটামিন 'এ'-র প্রয়োজন সে সম্পর্কে আমাদের ধারনা অপ্রতুল। জনস্বাস্থ্য বিশেষজ্ঞরা এই গবেষনালব্ধ তথ্যকে কাজে লাগিয়ে বিশ্বের বিভিন্ন স্থানে ভিটামিন 'এ'-র অভাব দূর করার জন্য প্রয়োজনীয় পদক্ষেপ নিতে পারবেন।

গোপনীয়তা ঃ

এই গবেষনায় প্রাপ্ত তথ্য শুধুমাত্র আই, সি, ডি,ডি, আর,বি ও ইউনিভার্সিটি অফ ক্যালিফোর্নিয়া ডেভিসে রক্ষিত থাকবে। কোন আইনগত কারনে প্রয়োজন না হলে আপনার নাম ও অন্যান্য ব্যক্তিগত তথ্যসমূহ সম্পূর্ণ গোপন রাখা হবে।

মূল্য/ক্ষতিপূরন ঃ

এই গবেষনার জন্য আমরা আপনার কাছে সময় চাইবো। গবেষনায় অংশগ্রহনের জন্য আপনাকে কোন খরচ বহন করতে হবে না। গবেষনা ক্ষেত্রে আপনি সকাল ৭টা থেকে সন্ধা ৭টা পর্যন্ত সময় দেয়ার জন্য আপনাকে আর্থিক ক্ষতিপূরন দেয়া হবে।

অংশগ্রহণকারীর সহি _____

সুনির্দিষ্টভাবে বলতে গেলে, আপনাকে প্রতিদিন ১৭০.০০ টাকা হারে ১২০ দিনের ক্ষতিপূরন দেয়া হবে। প্রতি ১০ দিন পর পর আপনাকে ১,৭০০ টাকা করে এই ক্ষতিপূরন পরিশোধ করা হবে। যদি গবেষনা প্রক্রিয়ায় আপনার কোন শারীরিক ক্ষতি হয় তাহলে আপনাকে বিনামূল্যে চিকিৎসা করা হবে। আই,সি,ডি,ডি, আর, বি বা ইউনিভার্সিটি অফ ক্যালিফোর্নিয়া ডেভিস এছাড়া অন্য কোন ধরনের ক্ষতিপূরন দিতে অপারগ।

গবেষনায় অংশগ্রহন প্রত্যাহার করার অধিকার ঃ

এই গবেষনায় সম্পূর্ন স্বতঃস্ফুর্তভাবে অংশগ্রহন করার জন্য আপনাকে আহবান করা হচ্ছে। আপনি গবেষনায় অংশগ্রহনে সম্মত হলেও গবেষনা কার্যক্রম শুরু হবার পর আপনার নাম প্রত্যাহার করতে পারেন।

প্রশ্নোত্তর ঃ

আপনার কোন প্রশ্ন থাকলে অনুগ্রহ করে জিজ্ঞাসা করুন। আপনার মনে যদি পরেও কোন প্রশ্ন জাগে সেক্ষেত্রে আপনার প্রশ্নের উত্তর ডাঃ জামিল, ডঃ হাস্কেল, ডাঃ ব্রাউন বা তাদের অধ্যস্তন কর্মীরা দিবেন। ডাঃ জামিলের ঠিকানা আই,সি,ডি,ডি,আর, বি মহাখালী, ঢাকা। ফোন নং - ৮৮১১৭৫১/২৩৩৩। ডঃ হাস্কেল ও ডাঃ ব্রাউনের ঠিকানা ঃ 3217 MEYER HALL, UC DAVIS, DAVIS, CA 95616, Tel. 001-530-752-1992.

সম্মতি ঃ

আপনার নীচের স্বাক্ষরটি এই অর্থ বহন করবে যে আপনি আপনার গবেষনায় অংশগ্রহন করছেন এবং আপনি উপরে বর্নিত তথ্যসমুহ এবং গবেষনায় অংশগ্রহনকারী হিসাবে আপনার অধিকার সম্পর্কে অবগত আছেন।

অংশগ্রহণকারীর নাম : _____

অংশগ্রহণকারীর স্বাক্ষর : _____ তারিখ : _____

গবেষকের নাম : _____

গবেষকের স্বাক্ষর : _____ তারিখ : _____

সাক্ষীর নাম : _____

সাক্ষীর স্বাক্ষর : _____ তারিখ : _____

আপনাকে স্বাক্ষর ও তারিখ সহকারে এই দলিলের একটি কপি সংরক্ষন করার জন্য দেয়া হবে। এ ছাড়া আপনাকে গবেষণা কাজে অংশগ্রহনকারী ব্যক্তির অধিকার সম্পর্কিত তথ্যও দেয়া হবে।

অংশগ্রহণকারীর সহি _____

(সম্মতি পত্র ২)

"একটি গবেষনায় অংশগ্রহন করার জন্য সম্মতি পত্র"

ইউনিভার্সিটি অফ ক্যালিফোর্নিয়া ডেভিস ও আই.সি.ডি.ডি.আর,বি : সেন্টার ফর হেল্‌থ এন্ড পপুলেশন রিসার্চ

গবেষনার নাম ঃ প্রাপ্তবয়স্ক পুরুষের জন্য ভিটামিন 'এ'-র প্রয়োজনীয়তা নির্ণয়

গবেষকদের নাম, বিভাগ, ফোন নং ঃ
ডাঃ কাজী মোঃ আসিফ জামিল, সি.এস.ডি, আই.সি.ডি.ডি.আর,বি, ফোন- (৮৮০-২) ৮৮১১৭৫১ এক্স ২৩৩৩
ডঃ মার্জোরি হাস্কেল, পুষ্টিবিদ্যা, ইউ,সি,ডেভিস (০০১) ৫৩০-৭৫৪-৭৪১৫;
ডাঃ কেনেথ ব্রাউন, পুষ্টিবিদ্যা, ইউ,সি,ডেভিস (০০১) ৫৩০-৭৫২-১৯৯২

উদ্দেশ্য ঃ
আপনাকে একটি গবেষনায় অংশগ্রহন করার জন্য আহ্বান করা হচ্ছে। বাংলাদেশের আই,সি,ডি,ডি, আর,বি ও ইউনিভার্সিটি অফ ক্যালির্ফোনিয়া ডেভিসের গবেষকরা একজন ব্যক্তির দৈনিক চাহিদা মিটানোর জন্য তার খাদ্যে কতটুকু পরিমান ভিটামিন 'এ' থাকা উচিত তা নির্ণয় করার জন্য এ গবেষনা চালাবেন।

পদ্ধতি ঃ
আপনি যদি স্বেচ্ছায় এই গবেষনায় যোগ দিতে চান তাহলে আপনাকে আগামী চার মাস আই,সি,ডি,ডি, আর, বি ক্যাফেটেরিয়াতে যে খাবার পরিবেশন করা হবে তার সবটুকু খেতে বলা হবে। আপনাকে প্রতিদিন সকাল ৭টার সময় গবেষনা ক্ষেত্রে উপস্থিত হতে হবে এবং সন্ধা ৭টা পর্যন্ত সেখানে আমাদের তত্ত্বাবধানে থাকতে হবে। এসময় আপনাকে সকালের নাস্তা, দুপুর ও রাতের খাবার এবং অন্য সময়ে হালকা নাস্তা পরিবেশন করা হবে। এখানে আপনার চিত্ত বিনোদনের জন্য খেলাধূলা ও সংবাদ পত্র পড়ার ব্যবস্থা থাকবে। আপনার জন্য বাইরে ব্যায়াম করার ব্যবস্থা ও গবেষনা ক্ষেত্রে বিশ্রাম নেয়ার ব্যবস্থা থাকবে।

খাবার সরবরাহের পাশাপাশি আপনাকে প্রতিদিন ভিটামিন ও খনিজ লবন পরিবেশন করা হবে। আপনাকে প্রতিদিন যেসব খাবার, ভিটামিন ও খনিজ লবন সরবরাহ করা হবে তাতে আপনার শরীরের ভিটামিন 'এ' ছাড়া অনান্য সকল পুষ্টির চাহিদা পূরন হবে। আপনাকে রাত্রে বাসায় ফিরার পর শুধুমাত্র পানি ছাড়া অন্যান্য সকল খাবার ও পানীয় গ্রহন করা থেকে বিরত থাকার জন্য অনুরোধ জানানো হবে। ক্যাফেটেরিয়াতে তিন সপ্তাহ খাওয়া দাওয়া করার পর আপনাকে পরবর্তী দুই মাস ধরে দুপুর ও রাতের খাবারের সঙ্গে প্রতিবেলায় একটি করে দিনে দুইটি ক্যাপসুল খেতে দেয়া হবে। এ ক্যাপসুলে বিভিন্ন মাত্রার, যেমন ২০০, ৫০০, ৮০০, ১১০০, ১৪০০, ১৭০০, ২১০০ বা ২৩০০ মাইক্রোগ্রাম পরিমান ভিটামিন 'এ' থাকবে। আপনাকে কোন মাত্রার ভিটামিন 'এ' দেয়া হবে তা লটারীর মাধ্যমে নির্ধারন করা হবে। এই দুইমাস ধরে আপনাকে ক্যাফেটেরিয়াতে যে খাবার পরিবেশন করা হবে তার সবটুকু খেতে বলা হবে। দুই মাস অতিক্রান্ত হবার পর আরও পাঁচ সপ্তাহ ধরে আপনাকে ক্যাফেটেরিয়াতে খেতে হবে - তবে এই সময়ে কোন ভিটামিন 'এ' ক্যাপসুল খেতে দেয়া হবে না।

বিকল্পঃ
বিকল্প উপায় হচ্ছে গবেষণায় অংশগ্রহণ না করা।

গবেষনার অন্যান্য প্রক্রিয়াগুলির বিবরন নীচে দেয়া হলো :

১। গবেষনা শুরু হবার প্রায় চার সপ্তাহ আগে থেকে আপনাকে চারবার (প্রতিবারে ৬০ মিঃ গ্রাম মাত্রার) ভিটামিন 'এ' ক্যাপাসুল খেতে দেয়া হবে যাতে আপনার শরীরে ভিটামিন 'এ'-র মজুদ বৃদ্ধি পায়। আপনাকে পাঁচদিন পর পর ভিটামিন 'এ'-র এই চারটি ডোজ খেতে দেয়া হবে। এই চার সপ্তাহ সময়ে আপনি আপনার স্বাভাবিক কার্যক্রম

উপকারিতা ঃ

আমাদের এই গবেষনার ফলাফল সামগ্রিকভাবে সমাজের সবার উপকারে আসবে। কারন শরীরের চাহিদা মিটানোর জন্য ঠিক কি পরিমান ভিটামিন 'এ'-র প্রয়োজন সে সম্পর্কে আমাদের ধারনা অপ্রতুল। জনস্বাস্থ্য বিশেষজ্ঞরা এই গবেষনালব্ধ তথ্যকে কাজে লাগিয়ে বিশ্বের বিভিন্ন স্থানে ভিটামিন 'এ'-র অভাব দূর করার জন্য প্রয়োজনীয় পদক্ষেপ নিতে পারবেন।

গোপনীয়তা ঃ

এই গবেষনায় প্রাপ্ত তথ্য শুধুমাত্র আই, সি, ডি,ডি, আর,বি ও ইউনিভার্সিটি অফ ক্যালিফোর্নিয়া ডেভিসে রক্ষিত থাকবে। কোন আইনগত কারনে প্রয়োজন না হলে আপনার নাম ও অন্যান্য ব্যক্তিগত তথ্যসমূহ সম্পূর্ণ গোপন রাখা হবে।

মুল্য/ক্ষতিপূরন ঃ

এই গবেষনার জন্য আমরা আপনার কাছে সময় চাইবো। গবেষনায় অংশগ্রহনের জন্য আপনাকে কোন খরচ বহন করতে হবে না। গবেষনা ক্ষেত্রে আপনি সকাল ৭টা থেকে সন্ধা ৭টা পর্যন্ত সময় দেয়ার জন্য আপনাকে আর্থিক ক্ষতিপূরন দেয়া হবে। সুনিদিষ্টভাবে বলতে গেলে, আপনাকে প্রতিদিন ১৭০.০০ টাকা হারে ১২০ দিনের ক্ষতিপূরন দেয়া হবে। প্রতি ১০ দিন পর পর আপনাকে ১,৭০০ টাকা করে এই ক্ষতিপূরন পরিশোধ করা হবে। যদি গবেষনা প্রক্রিয়ায় আপনার কোন শারীরিক ক্ষতি হয় তাহলে আপনাকে বিনামূল্যে চিকিৎসা করা হবে। আই,সি,ডি,ডি, আর, বি বা ইউনিভার্সিটি অফ ক্যালিফোর্নিয়া ডেভিস এছাড়া অন্য কোন ধরনের ক্ষতিপূরন দিতে অপারগ।

গবেষনায় অংশগ্রহন প্রত্যাহার করার অধিকার ঃ

এই গবেষনায় সম্পূর্ন স্বতঃস্ফুর্তভাবে অংশগ্রহন করার জন্য আপনাকে আহবান করা হচ্ছে। আপনি গবেষনায় অংশগ্রহনে সম্মত হলেও গবেষনা কার্যক্রম শুরু হবার পর আপনার নাম প্রত্যাহার করতে পারেন।

প্রশ্নোত্তর ঃ

আপনার কোন প্রশ্ন থাকলে অনুগ্রহ করে জিজ্ঞাসা করুন। আপনার মনে যদি পরেও কোন প্রশ্ন জাগে সেক্ষেত্রে আপনার প্রশ্নের উত্তর ডাঃ জামিল, ডঃ হাস্কেল, ডাঃ ব্রাউন বা তাদের অধ্যস্তন কর্মীরা দিবেন। ডাঃ জামিলের ঠিকানা আই,সি,ডি,ডি,আর, বি মহাখালী, ঢাকা। ফোন নং - ৮৮১১৭৫১/২৩৩৩। ডঃ হাস্কেল ও ডাঃ ব্রাউনের ঠিকানা ঃ 3217 MEYER HALL, UC DAVIS, DAVIS, CA 95616, Tel. 001-530-752-1992.

সম্মতি ঃ আপনার নীচের স্বাক্ষরটি এই অর্থ বহন করবে যে আপনি আপনার গবেষনায় অংশগ্রহন করছেন এবং আপনি উপরে বর্নিত তথ্যসমুহ এবং গবেষনায় অংশগ্রহনকারী হিসাবে আপনার অধিকার সম্পর্কে অবগত আছেন।

অংশগ্রহণকারীর নাম : _____

অংশগ্রহণকারীর স্বাক্ষর : _____ তারিখ : _____

গবেষকের নাম : _____

গবেষকের স্বাক্ষর : _____ তারিখ : _____

সাক্ষীর নাম : _____

সাক্ষীর স্বাক্ষর : _____ তারিখ : _____

আপনাকে স্বাক্ষর ও তারিখ সহকারে এই দলিলের একটি কপি সংরক্ষন করার জন্য দেয়া হবে। এ ছাড়া আপনাকে গবেষণা কাজে অংশগ্রহনকারী ব্যক্তির অধিকার সম্পর্কিত তথ্যও দেয়া হবে।

অংশগ্রহণকারীর সহি _____

Figure 1. Change in vitamin A pool size following 60-75 days of consumption of 0-3 mg retinol/d

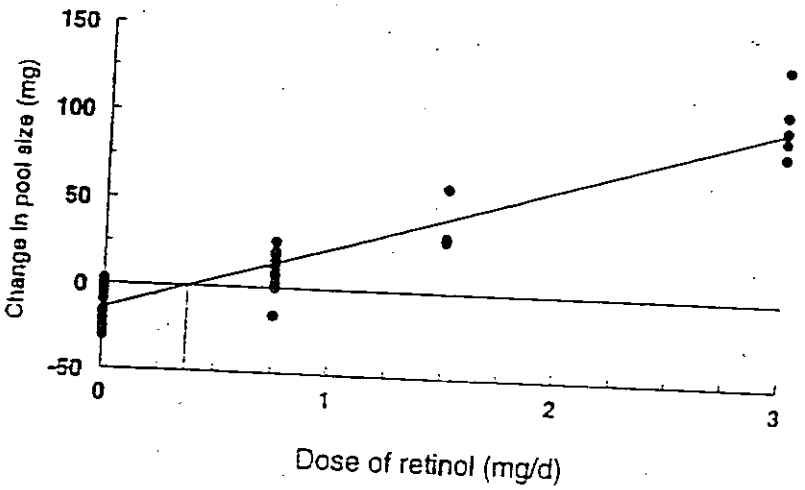
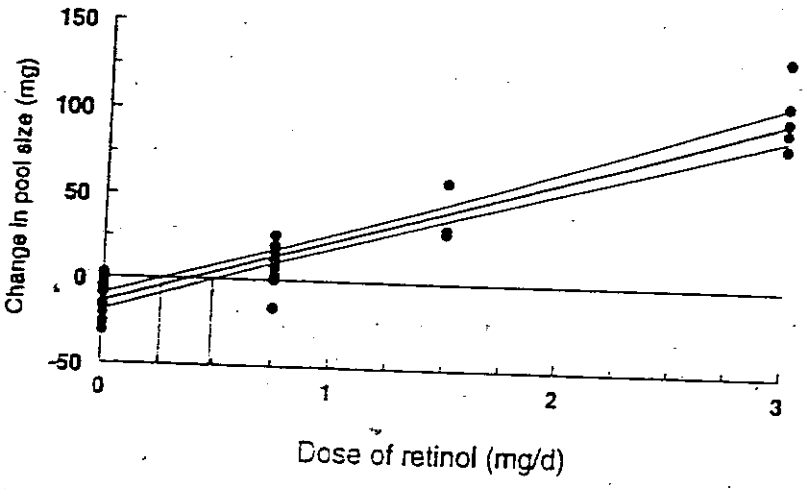


Figure 2. Change in vitamin A pool size following 60-75 days of consumption of 0-3 mg retinol/d



Linear regression line with 95% confidence interval

Figure 3. Study Design

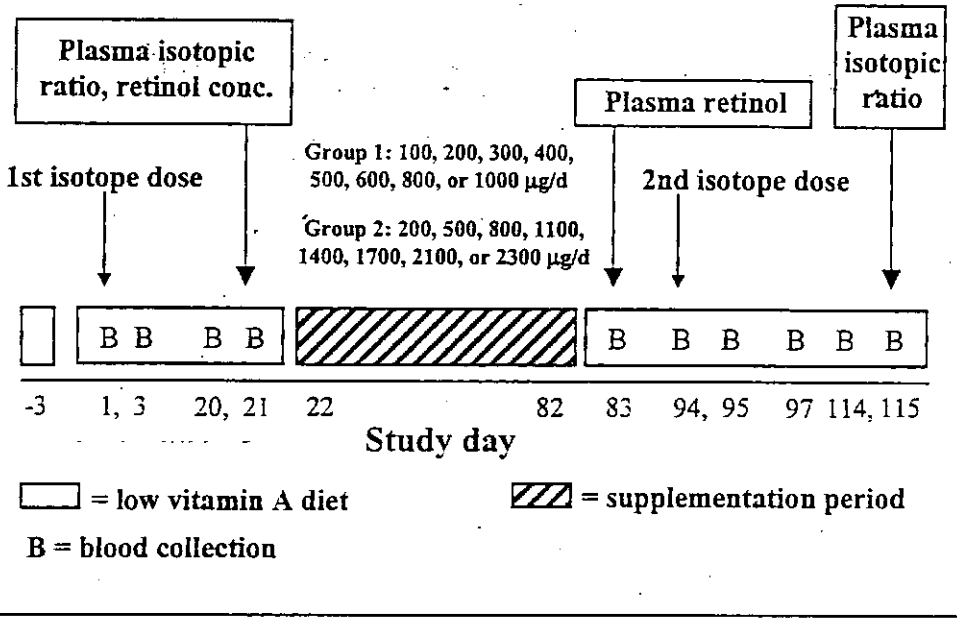
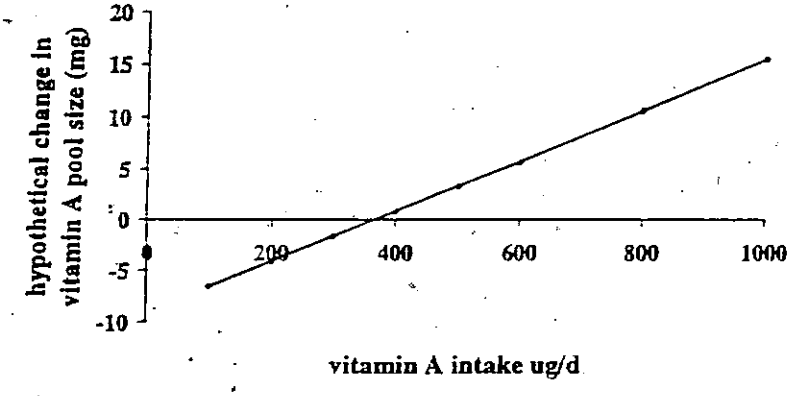
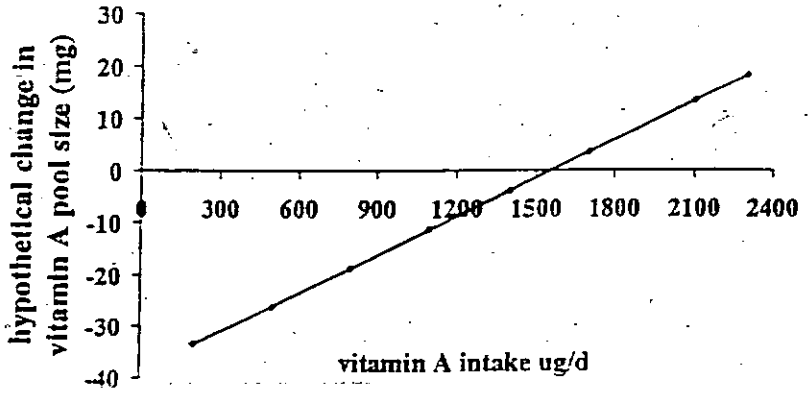


Figure 4. Hypothetical change in vitamin A pool size vs daily vitamin A intake in subjects with low initial vitamin A pool size



Assumptions: Mean initial pool size is 30 mg, vitamin A retention is 50% and the fractional catabolic rate is 0.5%/d

Figure 5. Hypothetical change in vitamin A pool size vs daily vitamin A intake in subjects with high initial vitamin A pool size



Assumptions: mean vitamin A pool size is 130 mg initially, vitamin A retention is 50% and the fractional catabolic rate is 0.5%/d

Table 1. Comparison of estimated change in pool size and theoretical change in pool size from previous studies

Daily vitamin A intake mg/d	Estimated change in vitamin A pool size (mg)	Theoretical change in vitamin A pool size (mg)	Ratio of estimated to theoretical change in pool size
0.75	18.8	18.5	1.02
1.5	58.3	54.0	1.08
3.0	125.8	107.8	1.17

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 judgment of the individual panel members. This panel summary reflects the consensus opinion of the panel regarding your proposal.

Proposal #: 0201006
PI: Haskell

Title: Estimation of Vitamin A Requirement of Adult Males

POSITIVE ASPECTS OF PROPOSAL:
The Food and Nutrition Board has identified research on the vitamin A requirement as a high priority. The study is well designed, and the investigators have extensive experience. It is good to do the study in Bangladesh where diets are naturally low in vitamin A and depletion of subjects is not required; this does not pose the logistical or ethical problems that depletion of subjects with higher normal intakes would do. Collaboration with Bangladeshi colleagues is well established.

NEGATIVE ASPECTS OF PROPOSAL:
The proposal states that 2 subjects/dose level are needed, and the protocol calls for 4/group, but no actual power analysis was done. The panel presumes that the *n* is based on the investigators' experience with similar studies.

SYNTHESIS COMMENTS:
The panel agreed this is an important project and suggestions for improvement were all relatively minor (see individual reviews). This work will provide data that will put recommendations for vitamin A intake on a modern scientific footing. The panel recommends funding this project with the highest level of enthusiasm.

Proposal Ranking

Your proposal was ranked #6 out of 111 proposals considered for funding this year. Your proposal was ranked in the following category:

Proposals Recommended for Funding

- ■ Outstanding (7.2%)
- ☐ High Priority (13.5%)
- ☐ Medium Priority - Upper (15.3%)
- ☐ Medium Priority - Middle (10.8%)
- ☐ Medium Priority - Lower (10.8%)
- ☐ Low Priority (17.1%)

Proposals Not Recommended for Funding

- ☐ Some Merit (13.0%)
- ☐ Do Not Fund (5.4%)

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Competitive Research Grants and Awards Management
National Research Initiative (NRI) Competitive Grants Program

PROPOSAL REVIEW SHEET

Proposal No.	Investigator
1006	Haskell
Please return to: Improving Human Nutr. 31.0	

SEE GUIDELINES FOR REVIEW

Comments: *(If needed, use additional sheets)* NOTE: Reviews, with reviewers' names removed, will be sent to applicants.

(2ndry) M. Haskell
Application # 02-01006

The purpose of this research proposal is to investigate vitamin A requirements. The PI has proposed to determine the vitamin A requirements in Bangali men with low and high initial vitamin A body pool size. The PI has hypothesized that the level of daily intake at which vitamin A pool size is maintained is a good estimation of daily vitamin A requirements. The proposal to better define vitamin A requirements based on maintaining the body's pool size and providing a good basis for the vitamin A requirement is needed. The information generated from this study will help to better define vitamin A requirements. The PI's have used the isotope ratio dilution techniques in their earlier studies to determine the β-carotene conversion to vitamin A and have planned to use the isotope dilution technique in subjects from Bangladesh where the low vitamin A body pool size is already available in the population due their dietary habits.

The PI has proposed to recruit 32 Bengali men after screening for low vitamin A status and to test the administration of several different levels of dietary vitamin A (100 to 1000 ug/d and 200-2300 ug/d) supplementation over 60 days on the vitamin A body pool size. Each level of vitamin A supplementation will be tested in 4 subjects. Before and after supplementation, the body pool sizes will be measured by administration of [$^2H_4$]-retinyl acetate and [$^2H_8$]-retinyl acetate respectively. The low levels of supplementation (100-1000 ug/d) will be administered to individuals with small body pool size (30 mg) and the high levels supplementation (200-2300 ug/d) will be used in individuals with high body pool size of vitamin A.

The study is very interesting and will provide information that can be used in defining vitamin A requirements, which are currently based on the limited vitamin A repletion studies in the elimination of vitamin A deficiency symptoms. The study design is well thought out to eliminate potential problems in the interpretation of the results. The PI has also provided the hypothetical model for the determination of vitamin A requirements. The PI has used the proposed isotope dilution method before in earlier studies and has a good knowledge of vitamin A metabolism and determining the body pool size. In addition, using volunteers from the Bangladesh population, who by comparison to Americans have a low body pool size of the vitamin A, is another positive point of this

(2ndry) M. Haskell
Application # 02-01006

study, which does not require a vitamin A depletion period, which requires a long period of time. Overall, the study design is well planned and commendable. The investigators of this project have the necessary expertise and logistics in place to carry on the clinical and laboratory components of this research proposal to a successful completion.

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As indicated by the PI, current estimates of human vitamin A requirements are based on "very limited data from two studies that were conducted more than 25 years ago." Furthermore, one of those studies (that of Sauberlich et al) was never described adequately in the scientific literature; the one publication obviously was intended to be followed by a fuller explication. Thus we simply do not have adequate data for estimation of an EAR or its confidence limits.

This proposal intends to measure body vitamin A pool size (by use of the isotope dilution technique) in adult males who maintain defined, graded dietary vitamin A intake. It is anticipated that body vitamin A pool size will be a linear function of dietary intake, over the range of intake studied.

I perceive no ethical problems with this study; subjects will not be exposed to harmful compounds and apparently will not be unduly restrained. I agree that the subjects on the lowest levels of vitamin A intake are not expected to develop vitamin A deficiency during the period of the study; subjects on the highest level of intake are very unlikely to experience vitamin A toxicity. However, it may be appropriate to provide vitamin A supplementation at the end of the study, especially for those subjects on low vitamin A intakes, and to provide diet counseling.

The investigative team has considerable experience working in Bangladesh on similar studies, and has demonstrated expertise with the isotope dilution technique also.

I believe that this proposal provides a unique opportunity to more accurately estimate human vitamin A requirements, and thus represents a major contribution to US nutritional standards.

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Scientific merit of the proposal:
The proposal sets forth and clearly defines one basic objective: to determine vitamin A requirements in Bangladeshi men. The PD has worked for several years in Bangladesh and therefore the likelihood of successful implementation of this proposal is quite high.

Qualifications of proposed project personnel and adequacy of facilities:
The PD has several years of experience with the proposed methodology and the facilities and therefore should have no problems implementing this study. She has allowed 2.5 years to accomplish the objectives (2 experiments). This should allow ample time for sample analysis and manuscript preparation.

Relevance of the project to long-range improvement in and sustainability of U.S. agriculture:
This proposal fits the objective of improving human nutrition for optimal health by trying to define human requirements of vitamin A.

Weaknesses: It is unclear why the PD references the 1989 RDA for men (1000 µg) when a lower RDA was adopted in 2001 by the Institute of Medicine, i.e. RDA of 900 µg and 700 µg retinol equivalents for men and women, respectively. Does she agree with the higher 1989 value (p. 2)? Under data analysis, it is also unclear why she chooses a 0.65 plasma to liver ratio (rats) when her own work has shown that this value is 0.8 in Bangladeshi men (p. 10). Will she further explore the reasons why there is a 15% discrepancy through modeling of data?
Do vitamin supplements really contain 166% of the RDA for vitamin A now (p. 7)?
The major weakness of this proposal is that the PD will use Bangladeshi men to model vitamin A requirements of U.S. men. This reviewer feels that there should be a Bangladeshi arm and a U.S.-based arm in the experiments. Therefore, the true vitamin A requirements of adult males in the U.S. could also be demonstrated. Repleting marginally deficient men to mimic the typical U.S. adult man seems strange especially when the PD resides in the U.S. Utilization rates will certainly be different and environmentally affected. This reviewer also feels that 8 different dietary levels are excessive (p. 8). Why not use 6 levels with 5 individuals at each level? The PD has already found linearity with 4 levels of treatment (p. 13) and therefore should be able to define the line with 6 levels of intake. The inclusion of 5 individuals at each treatment is better statistically than 4. Also please provide justification why blood samples will be drawn on days 20 and 21 (p. 9). It is unclear why back-to-back blood sampling is required. Why not use a one-week and a three-week to try to determine if earlier time points can be used?

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Comments: (If needed, use additional sheet) NOTE: Reviews, with reviewers' names removed, will be sent to applicants.

Title: Estimation of the vitamin A requirement of adult males

This proposal to estimate the average daily vitamin A requirement by stable isotope technique is excellent in every respect and deserves the highest priority. It fits exactly the goals of the Improving Human Nutrition for Optimal Health Program. Current vitamin A requirement is based on very old and sparse data, and better information is urgently needed, both for developed countries, where the supply of vitamin A may be even excessive, and in countries grappling with vitamin A deficiency, due to poverty, overpopulation or dietary habits.

The proposed use of the paired deuterated retinol dilution technique to estimate total body vitamin A stores and their change in response to supplementation is the most modern, harmless and relatively non invasive method in vitamin A metabolism research. The authors are very familiar with the method and used it successfully in their research, confirming the results with laboratory analysis of liver biopsies, and publishing their findings (reference 5 and 6).
The experiments are very well planned and described in detail. It is very important that the study will be conducted in Bangladesh within a population already low in vitamin A stores. There will be no problems of unethical depletion of subjects to the point of deficiency (present in old studies), because the study period is short. On the contrary, the supplementation during study should improve the vitamin A status of the subjects. It is very encouraging that the research will be conducted in cooperation with Bangladeshi medical organization and many laboratory analyses will be performed there, which strengthens local research capabilities and self-sufficiency. The authors have previously completed a series of studies in this location. The facilities are fully prepared for the required task both in Bangladesh and in California. A minor quibble: on page 7, line 3 and 4 from bottom "...men will be screened for plasma retinol and plasma CRP concentration". The abbreviation "CRP" is not explained anywhere in the text (C-reactive protein?), nor is the purpose of its screening (presence of inflammation reducing levels of plasma retinol?) or the analytical method. Otherwise the proposal is very clearly written, with exemplary explanation of the background, preliminary work, objectives and methodology. The competence of the authors is obvious and it was a pleasure to read this carefully prepared proposal.

In view of their previous accomplishments in the area of vitamin A metabolism, the authors should succeed in their endeavor, obtaining data which will be used as gold standard to develop better recommendations for vitamin A requirements, or to confirm the existing ones, which are admittedly still on a shaky ground.

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Scientific Merit:
The purpose of this study is to determine an estimate for the vitamin A requirement for adult males. The PI's present a convincing argument that the current estimates for vitamin A requirement are from limited data from more than 25 years ago and that it is necessary to re-evaluate the current recommendations and compare them to estimates based upon a more updated technique, that of deuterated-retinol-dilution method. The previous determinations focused on preventing deficiency. The current method measures body pools of vitamin A. The objective of this research is to determine the amount of vitamin A required to maintain low or high vitamin A body pools. The PI's will screen men from Bangladesh who normally have a low dietary intake of vitamin A and correspondingly low pools. These subjects will be supplemented at various doses to determine the dose required to maintain the low body pool (objective 1). They also will administer a challenge dose of vitamin A to another group of Bangladeshi men to increase their body pools and administer increasing concentration of vitamin A to determine an appropriate dose to maintain the higher body pool (objective 2). The proposal is well written, straightforward, and the results of this research will likely have a significant impact. The research is feasible and likely to have a successful outcome, because these investigators have done similar type of work using the same techniques and the same sample population. There were few and minor comments. Overall, this is a very sound well done proposal.

For objective 2, the Bangladeshi men will be challenged dosed to create a higher pool to simulate the pool size in the typical American male. Why not carry out the study in adult American men?
Under Study Procedures (p8 top), what procedure will be used for selecting subjects to carry out objective 2? the same as in objective 1?
For round 2 (p8 para 3), are they going to replete these subjects as mentioned previously? - by what method?
Under Post-supplementation vitamin A pool size estimate (p9), how did these investigators determine that 10 days is an appropriate time for the pool stabilization period? - previous studies?
At the end of the study (p9, 8 lines from bottom) why are the subjects going to receive a high dose of vitamin A? - to replete their low stores? Are they going to continue to supplement these individuals? If not, the vitamin A body pools of these subject will eventually return to their initial low levels, thus rendering the activity pointless.
For Figure 4 and 5, it appears that the slopes are equivalent, but it is likely that the slope of the line for the initially low pools of vitamin A would be much steeper than one that was generated when the initial pool was much greater.

Investigator and Institutional Capabilities: Adequate
Relevance to Long-range Improvement in and Sustainability of U.S. Agriculture: Appropriate
Other Considerations: None

7

International Centre for Diarrhoeal Disease Research, Bangladesh
CENTRE FOR HEALTH AND POPULATION RESEARCH
Mail : ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh
Phone: 880-2-8811751-60, Telex : 642486 ICDD BJ
Fax : 880-2-8827116, 8812530, 8811568, 8826050, 9885657, 8811686, 8812529
Cable : Cholera Dhaka

6 November 2001

Kenneth H Brown, MD
Program in International Nutrition and
Department of Nutrition
University of California
Davis, CA 95616
USA
Fax: 530 752 3406

Dear Dr Brown

I am in receipt of your email communication of 6 November requesting a letter of collaboration with ICDDR,B for the next round of vitamin A-related studies.

The Centre would be happy to work with your group on the study "Estimation of vitamin A requirements of adult males". I look forward to receiving a copy of the proposal.

Regarding the proposed teleconference to discuss nutrition-related research priorities for UC Davis-ICDDR,B collaboration, I have passed your email to Peter Thorpe who should be responding to you in due course.

Regards

Yours sincerely

David A Sack MD
Director

International Centre for Diarrhoeal Disease Research, Bangladesh
CENTRE FOR HEALTH AND POPULATION RESEARCH
Mail : ICDDR, B, GPO Box 128, Dhaka-1000, Bangladesh
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Cable : Cholera Dhaka

November 10, 2002

To: Prof. Mahmudur Rahman
Chairman, ERC

From: Dr. Iqbal Kabir
Scientist, ICDDR,B & Member, ERC

Subject: **Review of the Protocol " Estimation of average vitamin A requirement of adult males.**

General Comments:

Thanks for sending the protocol for my review and comments. In brief, the protocol aims to study the daily requirement (RDA) of vitamin A in adult males. The investigators will use deuterated retinol (stable isotopes) dilution technique to estimate the pool size of vitamin A in the body store. This technique had already been utilized by the same group of investigators in the earlier studies at ICDDRB. This time the study will estimate the amount of vitamin A required daily to maintain vitamin A pool size in two groups of subjects; low vitamin A body store (mean of ~ 30 mg) and high vitamin A body store (mean of ~ 130 mg).

The study will be conducted in two rounds and 75 adult subjects (18-35 years of age) will screened for each round. 32 subjects will be selected on the basis of low level of plasma vitamin A concentration (< 35 ug/dl) and will be maintained with low vitamin A diet 60 days and different doses of vitamin A to estimate the dose responses. Similarly during the 2nd round 32 subjects will be selected and be given high vitamin doses to estimate the dose response. The findings of this study will useful to estimate the daily requirement of vitamin A in Bangladeshi adult males.

Specific Comments:
In general the protocol does not have any major ethical concern. These investigators have previously conducted similar studies at ICDDR,B utilizing the same methods. Although, they did not have baseline estimation of vitamin A pool size in those studies, hence, the rational for current study was justified. I understand, there should not be any problem with keeping adult males in low vitamin A diet for 4 months. However, some of the subjects may develop severe infection (dysentery, febrile illness, where vitamin depletion

and increased loss through gut and urine have been documented. It is therefore, advisable to drop the subject if anybody develop such severe infection during the study period. The amount of blood will be drawn at different time periods to estimate plasma retinol, and labeled vitamin A (10 ml at 10 days). During the 2nd round subjects will be given high dose of vitamin A. Although, it was mentioned that there is no major side effects in this non-toxic dose, but it is possible that vitamin A might have adverse effect with sub-clinical liver disease. It may be useful to screen for abnormal liver function test in these subjects at baseline.

1. Face sheet looks OK, except in point 2a) physical risk should be mentioned as there is some minor risks as mentioned in the consents forms.
2. Voluntary consent form for screening procedure: Both the English and Bangla consent forms provided details. However, I don't find any justification to draw 10 ml of blood to determine the plasma vitamin A concentration and CRP (i believe they mean, C-reactive protein). I have checked with the laboratory and found 2-3 ml of blood would be enough.
3. All others consent forms 1st and 2nd are similarly described in details, though in the Bangla consent forms it was not mentioned that, there is no personal and direct benefits to the subjects participating into this study.
4. In the consent forms; Costs/Compensation; last 4 line read; If you are injured as a direct result of research procedure, you will receive reasonably necessary medical treatment. However, no other form of compensation will be provided. I think the investigators should define what is *reasonable*, and also should compensate any other benefits; as the study does not have direct benefits to the subjects.

I personally think, this is an important study and will provide valuable scientific knowledge. With the suggested minor modification, the protocol may be considered for ethical approval.