

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. A.N. Alam  
Application No. 85-024P  
Title of Study "BIOAVAILABILITY OF IRON FROM BANGLADESHI MEALS".

Trainee Investigator (if any)  
Supporting Agency (if Non-ICDDR,B)  
Project status:  
(X) New Study  
( ) Continuation with change  
( ) No change (do not fill out rest of form)

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

- Source of Population:
- (a) Ill subjects  Yes  No
  - (b) Non-ill subjects  Yes  No
  - (c) Minors or persons under guardianship Yes  No
- Does the study involve:
- (a) Physical risks to the subjects Yes  No
  - (b) Social Risks Yes  No
  - (c) Psychological risks to subjects  Yes  No
  - (d) Discomfort to subjects  Yes  No
  - (e) Invasion of privacy Yes  No
  - (f) Disclosure of information damaging to subject or others Yes  No
- Does the study involve:
- (a) Use of records, (hospital, medical, death, birth or other)  Yes  No
  - (b) Use of fetal tissue or abortus Yes  No
  - (c) Use of organs or body fluids  Yes  No
- Are subjects clearly informed about:
- (a) Nature and purposes of study  Yes  No
  - (b) Procedures to be followed including alternatives used  Yes  No
  - (c) Physical risks Yes  No
  - (d) Sensitive questions Yes  No
  - (e) Benefits to be derived  Yes  No
  - (f) Right to refuse to participate or to withdraw from study  Yes  No
  - (g) Confidential handling of data  Yes  No
  - (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure Yes  No

- 5. Will signed consent form be required:
    - (a) From subjects  Yes  No
    - (b) From parent or guardian (if subjects are minors) Yes  No
  - 6. Will precautions be taken to protect anonymity of subjects  Yes  No
  - 7. Check documents being submitted herewith to Committee:
    - Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
    - Protocol (Required)
    - Abstract Summary (Required)
    - Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
    - Informed consent form for subjects
    - Informed consent form for parent or guardian
    - Procedure for maintaining confidentiality
    - Questionnaire or interview schedule \*
- \* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
  2. Examples of the type of specific questions to be asked in the sensitive areas.
  3. An indication as to when the questionnaire will be presented to the Cttee. for review.

(PTO)

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

Dr. Alam  
Principal Investigator

\_\_\_\_\_  
Trainee

85-024P  
25-7-85

SECTION 1 - RESEARCH PROTOCOL

(Pilot protocol)

1. TITLE: BIOAVAILABILITY OF IRON FROM  
BANGLADESHI MEALS.
  
2. PRINCIPAL INVESTIGATOR: Dr. A.N. Alam  
CO-INVESTIGATORS: Mr. M.A. Wahed  
Dr. M.A. Rashid  
Professor of Haematology, IPGM&R.  
Dr. M.M. Rahaman  
  
CONSULTANT: Dr. J.D. Cook  
(International Center for Control  
of Nutritional Anaemia, Kansas  
University Medical Center, Kansas,  
U.S.A.).
  
3. STARTING DATE: August 1985
  
4. COMPLETION DATE: January 1986
  
5. TOTAL INCREMENTAL COST: US \$ 1,550.00
  
6. SCIENTIFIC PROGRAMME: This protocol has been approved by  
the Nutrition Working Group.

Signature of Scientific Programme Head:

*Uyky Kalim*

Date:

23/7/1985

7. ABSTRACT SUMMARY:

Iron deficiency anaemia is a major public health problem in both Bangladesh and elsewhere although diet in most countries contains large amount of iron. The present study proposes to determine the bioavailability of iron from different types of Bangladeshi meals, tagged extrinsically with a small amount of radioactive iron, in ten healthy adult males and five anaemic adult male subjects. This will help in suggesting ways to improve iron nutrition in our population.

8. REVIEWS:

- (a) Research involving human subjects: -----
- (b) Research Review Committee: -----
- (c) Director: -----

SECTION II - RESEARCH PLAN

A. INTRODUCTION:

1. Objectives:

The aim of this study is to determine the bioavailability of iron from whole Bangladeshi diet.

2. Background.

Most nutritional anaemias in a population are due to iron deficiency<sup>1</sup>. This is attributed to a marginal intake of iron or more importantly to its low bioavailability. Dietary iron as it relates to iron uptake by the intestinal mucosal cell is divided into two distinct compartments: nonhaeme and haeme iron. Non-haeme iron derived from cereals, fruits, vegetables etc. constitute the larger component of dietary iron. Assimilation of this form of iron is determined largely by the extent to which it remains soluble as reduced ionic iron within the upper intestinal lumen. Because foods differ substantially in their content of factors that promote or inhibit iron solubility, the absorption of nonhaemē iron is greatly influenced by the nature of the meal<sup>2</sup>. In contrast, haeme iron derived from haemoglobin and myoglobin gains entry to the mucosal cell as an intact iron porphyrin complex and is relatively independent of other components of the meal. It forms a relatively minor part of total iron intake. Even in Western diets with high meat content, haeme iron usually accounts for only 10 to 15 per cent of the total daily iron intake.

In most developing countries, dietary iron intake may range from 15 to 30 mg<sup>3,4</sup>. However, people eat less of meat and fish, because of economic restraints. Although absorption of non-haeme iron is greatly affected by the iron status of the subject, this lack of dietary haeme iron could partly account for iron deficiency anaemia. The latter results in reduced work performance and thereby impose economic limitations on people whose livelihood depends on physical work. The main source of dietary iron in rural Bangladesh is from rice and only 1 to 2% of the iron in rice has been shown to be absorbed by normal subjects<sup>5</sup>. Dietary fibre with a modest inhibitory effect on iron assimilation<sup>6</sup> may partly contribute to low availability from Bangladeshi diet. Associated hookworm infestation may also create additional requirements for iron<sup>7</sup>. Iron absorption in the adult amounts to about 0.5-2 mg/day (equalling the daily loss) and this occurs in the small intestine (largely duodenum), apparently in the ferrous state. Iron may combine with intracellular storage proteins called ferritin and mucosal transferrin in the intestinal mucosal cells before being absorbed.

National Nutrition Surveys conducted in 1975-76<sup>8</sup> and 1981-82<sup>9</sup> revealed a high prevalence of anaemia (7 out of every 10 people are anaemic) in Bangladesh. A recent study<sup>10</sup> conducted in a rural area has shown anaemia to be present in about 35 to 50% of the population depending on age and sex groupings as classified by WHO and majority of them are iron deficient as determined by serum ferritin estimation. Low bioavailability of dietary iron may be responsible for the high prevalence of iron deficiency anaemia in this region. This, in turn, may decrease the work capacity of our economically underprivileged population.

An important recent advance in studies of food iron assimilation was the demonstration that iron absorption can be measured by simply adding a small amount of inorganic radioiron with the administered food (extrinsic tag) rather than biosynthetically incorporating the label<sup>11</sup>. It was possible to show that a common pool of nonheme or haeme iron is formed in the intestinal lumen by several foods ingested in the same meal and that absorption from this pool can like-wise be measured by extrinsic tagging<sup>12,13</sup>. Thus food iron absorption depends not on the food source but on the composite effect of factors in a meal that either block or promote iron availability, largely by affecting its solubility.

It is usually unnecessary to measure the absorption of haeme iron in Third World countries because it constitutes only a small portion of dietary iron and is little affected by the composition of the diet. Non-haeme iron absorption measurements are therefore usually sufficient specially in nutritional studies. The effect of subject-to-subject differences in iron status can be eliminated by performing as many as four separate absorption measurements using dual radioisotopes in the same individual. This will also allow comparisons to be made between different meals. Moreover, the inclusion of a radioiron reference dose (containing 3 mg ferrous iron, which represents the final common pathway by which all forms of dietary iron with the exception of haemoglobin are assimilated, and 30 mg ascorbic acid) measurements to characterize iron status of a subject is particularly important when evaluating iron availability from regional diets.

By measuring absorption in each subject both from meals and the reference dose, the results can be expressed as a regression line between the two absorption measurements. A difference in iron absorption between two meals can then be assessed by comparing the slopes of the regression lines relating food iron absorption to absorption of the reference dose. This implies that the ratio or mean absorption from a meal and from the reference dose is a measure of relative bioavailability of non-haeme iron in the meal. Because of the highly skewed distribution of percentage absorption, analysis is performed on the logarithmic scale using geometric mean<sup>14</sup>.

B. SPECIFIC AIMS:

In spite of relatively large intake of iron in the diet, a high incidence of iron deficiency anaemia has been observed in Bangladesh. The present study will aim at evaluating the possible inhibitory role of locally available diet in causing anaemia in the population.

C. METHODS AND PROCEDURE:

Ten apparently healthy iron-replete adult males and five anaemic subjects (iron deficiency will be confirmed by serum ferritin determination prior to selection for the study) between 18 - 50 years of age and without history of G.I. disorders, acute or chronic infection, or hematologic disease will be selected for the study. Informed consent will be obtained in every case explaining the risk and discomfort involved in the study. To avoid any imprecision when determining absorption from a single test meal, comparison will be made between absorption of iron from four separate test meals. Five ml of venous blood will be taken from the study subjects on the previous day for background radioactivity and conventional hematologic measurements, such as, haematocrit serum iron, TIBC and serum ferritin. Height and weight will also be taken. After an overnight fast, they will be given test meals (according to the meal plan) on two successive mornings tagged with either  $^{59}\text{Fe}$  or  $^{55}\text{Fe}$ . The subjects will be allowed to resume their normal diet 3 hours after the test meal. Absorption from meals tagged extrinsically with ferric chloride will be determined from the radioactivity (circulating red cell radioactivity) incorporated into blood obtained 14 days after the administration of the test meals. A further two absorption tests will be performed using  $^{59}\text{Fe}$  or  $^{55}\text{Fe}$  in the test meals offered on day 15 and 16 after overnight fast. Absorption of these test meals will be determined by measuring the rise in radioactivity in blood sample obtained on day 30.



The test meals will be as follows:

Meal Plan:

Day - 1	Day - 2	Day - 15	Day - 16
200 gm rice 100 gm lentils 35-40 gm vegetables (potatoes, cauliflower, and green bean, carrots)  Water ad libitum to drink	200 gm wheat chapatti and 300-40 gm vegetables as on day 1  Water ad-libitum to drink	250 g samilina 120 ml whole milk 24 g sugar 14 g butter	<p><u>Reference dose:</u> 3 mg FeSO<sub>4</sub> 7H<sub>2</sub>O is dissolved in 1 ml 0.01 N HCl for each volunteer. The isotope is then added is to the solution.</p> <hr/> <p>18.9 mg ascorbic acid is dissolved in 50 ml water</p> <hr/> <p>One ml of isotope solution will be added to ascorbic acid solution prior to administration to the subject.</p>
+ 1.5 $\mu$ ci <sup>55</sup> FeCl <sub>3</sub>	+ 0.5 $\mu$ ci <sup>59</sup> FeCl <sub>3</sub> with 0.1 mg carrier FeCl <sub>3</sub>	+ 1.5 $\mu$ ci <sup>55</sup> FeCl <sub>3</sub> with 3 mg carrier FeCl <sub>3</sub>	+ 0.5 $\mu$ ci <sup>59</sup> FeCl <sub>3</sub>
Radioiron will be added to one of the food items immediately before it is offered to the subject.			Will be given as a drink.

The total dose of radioactivity administered to each subject will be 3  $\mu$ ci <sup>55</sup>Fe and 1  $\mu$ ci <sup>59</sup>Fe and a total of 15 ml of venous blood will be drawn from each subject. Assuming a maximum mean absorption of 10 percent of the administered test dose, the above radiation doses will be less than one percent of the maximal permissible doses for occupationally exposed persons <sup>15</sup>.

Calculation of iron absorption from incorporated blood radio-  
activity requires (i) an estimate of total blood volume from height  
and weight using the following formula:

$$B.V. = 28.5 \times Ht \text{ (in cm)} + 31.6 \times Wt \text{ (in kg)} - 2820$$

and an (ii) estimate of the proportion of absorbed radioactivity  
incorporated into circulating red cells (normally 80-90%).

Measurement of blood radioactivity will be done at the Hematology  
Research Laboratory, Kansas University Medical Center, USA. In  
principle, the samples of whole blood are pipetted into Kjeldahl  
flasks and are then prepared by wet digestion for liquid scintillation  
counting using the method of Eakins and Brown<sup>16</sup>. For each administered  
dose of radio-iron absorption is calculated as follows:

$$\text{Absorbed counts (X)} = \text{CPM blood} \times \text{blood volume}$$

$$\text{Administered counts (X)} = \text{CPM standard} \times 500$$

$$\text{Absorption (\%)} = \frac{\text{Absorbed counts (X)}}{\text{Administered counts (X)}} = 100 \times F$$

where 'F' is the factor to adjust for incomplete incorporation of  
absorbed radioactivity into circulating whole blood.

D. COLLABORATIVE ARRANGEMENTS:

This will be part of a multinational study on iron absorption  
to be done in collaboration with the International Centre for Control  
of Nutritional Anaemia (ICCNA) at the University of Kansas Medical  
Center. Iron absorption study was carried out in healthy American  
volunteers using Bangladeshi diet as the first part of the collabora-  
tive study.

E. SIGNIFICANCE:

The proposed study will help us in establishing ways to improve iron nutrition either by institution of remedial measures such as, supplementation, fortification or by adding substances to the diet that would render the iron more available for absorption.

REFERENCES:

1. Report of a WHO Group of Experts (1972) WHO Tech Rep Series No. 503 : 1-29.
2. Mayrisse M et al (1968) Am J Clin Nutr 21 : 1175
3. Apte SV and Venkatachalam PS (1962) Ind J Med Res 50 : 516
4. Hassan N and Ahmad K (1984) Food and Nutrition Bulletin 6 : 34
5. Aung-Thun-Batu et al (1972) Am J Clin Nutr 25 : 210.
6. Cook JD et al (1983) G.E. 85 : 1354
7. Bothwell TH et al (1979) Iron metabolism in man, Oxford. Blackwell 7 : 43.
8. Nutrition Survey of Rural Bangladesh, 1975-76 INFS, Dhaka, 1977.
9. Nutrition Survey of Rural Bangladesh, 1981-82 INFS, Dhaka.
10. Alam AN et al. Unpublished observation
11. Cook JD et al (1972). JCI, 51 : 805
12. Layrisse M et al (1972). Am J Clin Nutr 25 : 401
13. Hallberg L and Bjorn - Rasmussen E (1972). Scand J Haematol 9:193.
14. Cook JD et al (1969) Blood. 33 : 421
15. Robertson JS et al (1983). J.Nucl Med 24 : 339.
16. Eakins and Brown (1966) Int J Appl Rad Isotopes 17 : 391

SECTION III - BUDGET1. Personnel services:

<u>Name</u>	<u>Position</u>	<u>% effort</u>	<u>Project requirement</u>	
			<u>Taka</u>	<u>Dollar</u>
Dr. A.N. Alam	Principal Investigator	20%	144,000	5760.00
Mr. M.A. Wahed	Co-Investigator	10%	36,000	1440.00
Dr. M.A. Rashid (Prof. of Haematology, IPGM&R)	do		-	-
Dr. M.M. Rahaman	do		-	-
Dr. J.D. Cook	Consultant		-	-
Sub Total			=	US \$ 7,200.00

2. Supplies and materials

<sup>55</sup> Fe Cl <sub>3</sub>	..	400.00
<sup>59</sup> Fe Cl <sub>3</sub>		
Source: Amersham, U.K.		
Biochemical tests:	..	263.00
Ferritin		
Serum iron		
TIBC		
Haematological test: HCT		

3. Equipment	- nil	
4. ICDDR,B transport	- nil	
5. Patient hospitalisation 5 X 6 X 200	..	6,000 240.00
6. Outpatient care	- nil	
7. Transportation of patients and things	..	280.00
8. Travel	- nil	
9. Rent, communication, utilities	- nil	
10. Printing and publication	- nil	
11. Other contractual services	- nil	
12. Construction	-	

GRAND TOTAL = \$ 8383.00  
Incremental cost = \$ 1183.00  
31 % overhead cost = \$ 367.00

TOTAL US \$ = 1550.00

ABSTRACT SUMMARY FOR ETHICAL REVIEW COMMITTEE

1. Two groups of adult male subjects, ten apparently health volunteers and five anaemic subjects will be studied. Healthy volunteers will be selected from amongst ICDDR,B employees and five anaemic subjects will be selected from patients reporting to IPGM&R.
2. Blood will be obtained by venepuncture which may cause little discomfort. Small amount (total of 4  $\mu$ ci) of radioisotopes in the form of ferric chloride will be offered with food. This amount is far less than the recommended dose for human experimentation (a copy of relevant certificate is enclosed).
3. All possible care will be taken and trained personnel will draw venous blood to cause minimal pain.
4. Only hospital identification numbers of the study patient will be used.
5. Informed written consent will be obtained from study subjects.
6. The study will involve obtaining information about clinical history only and it will take less than 5 minutes to take the interview of either the volunteer subject or the patient.
7. The study patients will be treated adequately for anaemia at the IPGM&R.
8. This protocol requires the use of hospital records and body fluid.



# INSTITUTE OF NUCLEAR MEDICINE

BANGLADESH ATOMIC ENERGY COMMISSION

P. O. BOX NO. 158, RAMNA


DACCA-2

Cable : BANGLATOM  
Phone : 502757  
505194-8/95

Date : 20.5.1985

No. \_\_\_\_\_

This is to certify that the use of 3 microcurie radioiron - 59 and 1 microcurie of radioiron - 55 for iron absorption studies in human being is much below the maximum permissible level and is within safety limit recommended by I.C.R.P.

  
( Kamaluddin Ahmed )  
Director.

CONSENT FORMA STUDY OF FOOD IRON BIOAVAILABILITY

There is high incidence of iron deficiency anaemia in Bangladesh inspite of abundance of iron in the Bangladeshi diet. It may be due to low bioavailability of iron from those diets. ICDDR,B in collaboration with Kansas Medical Center, USA has undertaken a study to investigate the causes of such low bioavailability in healthy and anaemia adult males. This study is expected to reveal useful information to initiate improvement of iron nutrition in the population either by iron supplementation to the diet or possibly by adding substances to the diet that should render the iron more available for absorption. We would like you to participate in the study.

You will be offered diets after overnight fast according to the following schedule:

First phase (on two successive days):

Day - 1. Rice, lentils, vegetables	The meals will contain isotopes of iron (1.5 $\mu$ ci and 0.5 $\mu$ ci respectively).
Day - 2. Chapati and vegetables	

Second phase (on two successive days)

After 14 days of 1st phase, the following diet with isotopes of iron will be offered:

Day - 3. Samolina, milk, sugar with 1.5 $\mu$ ci	<sup>55</sup> FeCl <sub>3</sub>
Day - 4. Ascorbic acid with 0.5 $\mu$ ci	<sup>59</sup> FeCl <sub>3</sub>

For laboratory examinations 5 ml of venous blood before first phase and at the end of each phases will be drawn. This amount of blood (a total of 15 ml) drawn and the dose of isotope will not cause any problem to you.

You may not have to stay in the hospital. But we will like you to come to the hospital in the morning after overnight fast for required investigations. We will provide you with proper treatment if anaemia is found. To protect your personal social status, we will maintain the confidentiality of data.

If you agree to participate in the study, please put your signature or thumb impression below. You have every right to ask any question about the study. Moreover, you may withdraw your consent at any time during the study.

\_\_\_\_\_  
Signature of the investigator

\_\_\_\_\_  
Signature/Left thumb impression of the volunteer.

Date: -----



স্মৃতি পত্র ।  
=====

বাংলাদেশ খাবারে লৌহজাতীয় পদার্থ প্রচুর স্বাক্ষর জৌহজাতীয় অভাব হেতু রক্ত শূন্যতা বিরাজ করছে। আমেরিকায় ক্যানসাস মেডিকেল সেকৌরের সহযোগীতায় আই, সি, ডি, ডি, আর, বি উহার কার্য অনুসন্ধানের জন্য এই গবেষণার পরিকল্পনা করেছে। এই গবেষণায় সুস্থ সবল এবং রক্ত শূন্যতায় ভুগছেন এমন পুরুষ ব্যক্তিদের অংশ গ্রহন আমরা আশা করি। এই গবেষণা জনসাধারণের মাঝে রক্ত শূন্যতা দূরীকরণের নানাহ ব্যবস্থা নির্ধারণে বিশেষভাবে সাহায্য করবে। গবেষণার অংশ হিসাবে নিম্নসূচী মোতাবেক আপনাকে খাবার দেওয়া হবে :

প্রথম পর্যায়ে :

পর পর দুইদিন খালিপেটে ডাল-ভাত-দই-মুগি  
এবং আটার রুটি ও দই

সাথে ০.৫ অথবা ১.৫ মাইক্রোকিউরি  
লৌহ আইসোটোপ দেওয়া হবে।

দ্বিতীয় পর্যায়ে :

প্রথম পর্যায়ের ১৪ দিন পর আবার খালি পেটে পর পর দুইদিন খাবার ও ২ মাইক্রোকিউরি আইসোটোপ দেওয়া হবে। পরিকার জন্য প্রথমে ৫ সি, সি রক্ত এবং উভয় পর্যায়ে আরো ১০ সি, সি, পরিমাণ রক্ত নেওয়া হবে। তাতে আপনার শরীরের কোন ক্ষতি হবে না।

আপনার হাসপাতালে অবস্থানে কোন বাধাবাধতা নাই। গবেষণা চলাকালে আপনার শরীরের-যাবতীয় যত্ন নেওয়া হবে। গবেষণার তথ্যাদীর গোপনীয়তা অবশ্যই রক্ষা করা হবে যাতে করে আপনার ব্যক্তিগত কোন ভাবে ক্ষতি না হয়।

এখানে উল্লেখ থাকে যে, আপনি গবেষণা সময়ে যে কোন বিষয় জানার এবং গবেষণা থেকে যে কোন সময়ে সমস্তি ক্ষতিহারের অধিকার রাখেন।

আপনি এই গবেষণায় অংশ গ্রহণ রাজী থাকলে নিচে স্বাক্ষর/টিপসহি করুন।

গবেষণাকারীর স্বাক্ষর

গবেষণায় অংশ গ্রহণকারীর স্বাক্ষর/টিপসহি

তারিখ : \_\_\_\_\_

তারিখ : \_\_\_\_\_