Attachment 1.
(FACE SHEET)

Revised

ETHICAL REVIEW CO[illegible]E, ICDDR,B.

Date 31.12.95

Principal Investigator George J. Fuchs, M[illegible]rainee Investigator (if any) _____

Application No. 95-033

Supporting Agency (if Non-ICDDR,B) _____

Title of Study "Effect of Iron Supplementation on Growth and Intestinal Permeability of Iron-replete and Iron-Deplete Children".

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).

1. Source of Population:
   (a) Ill subjects — Yes (No)
   (b) Non-ill subjects — (Yes) No
   (c) Minors or persons under guardianship — (Yes) No
2. Does the study involve:
   (a) Physical risks to the subjects — Yes (No)
   (b) Social Risks — Yes (No)
   (c) Psychological risks to subjects — Yes (No)
   (d) Discomfort to subjects — (Yes) No
   (e) Invasion of privacy — Yes (No)
   (f) Disclosure of information damaging to subject or others — Yes (No)
3. Does the study involve:
   (a) Use of records, (hospital, medical, death, birth or other) — Yes (No)
   (b) Use of fetal tissue or abortus — Yes (No)
   (c) Use of organs or body fluids — (Yes) No
4. Are subjects clearly informed about:
   (a) Nature and purposes of study — (Yes) No
   (b) Procedures to be followed including alternatives used — (Yes) No
   (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:
   N/A 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)
   N/A Informed consent form for subjects
   ✓ Informed consent form for parent or guardian
   N/A Procedure for maintaining confidentiality
   N/A 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.

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

[signature] MD
Principal Investigator

28/12/95
Trainee

## CHECK-LIST FOR SUBMISSION OF PROPOSALS TO THE RESEARCH REVIEW COMMITTEE (RRC)

**[Please tick (✓) the appropriate box]**

1. Has the proposal been reviewed, discussed and cleared at the Division level ?

   Yes [✓]

   No [ ]

   If the answer is 'NO', please clarify the reasons: _____

2. Has the proposal been peer-reviewed externally ?

   Yes [✓]

   No [ ]

   If the answer is 'NO', please explain the reasons: _____

3. Does the proposal address gender issues ?

   Yes [✓]

   No [ ]

   If the answer is 'NO', Please give the reasons.

4. Has a funding source been identified ?

   Yes [ ]

   No [✓]

   If the answer is 'YES', please indicate the name of the donor: _____

Contd... /

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5. Whether the proposal is a collaborative one ?

Yes ☑

No ☐

If the answer is 'YES', the type of collaboration, name and address of the institution and name of the collaborating investigator be indicated:

The hormone assays will be done in the laboratories of Dr. Chris Kelnar, University of Edinburgh, U.K. Drs Doherty and Cutting of University of Edinburgh will provide the knemometry and assist in data analysis.

6. Has the budget been cleared by Finance Division ?

Yes ☑

No ☐

If the answer is 'NO', reasons thereof be indicated: _____

7. Does the study involve any procedure employing hazardous materials, or equipments ?

Yes ☐

No ☑

If the answer is 'YES', fill the necessary form.

28/12/95
Date

Signature of the
Principal Investigator

**Title:** **Effect Of Iron Supplementation On Growth And Intestinal Permeability Of Iron-replete And Iron-deplete Children**

**Investigators:**

George Fuchs, MD, (P.I.), Clinical Sciences Division, ICDDR,B
M. Aminul Islam, MBBS, M.Sc., (Co-PI), Clinical Sciences Division, ICDDR,B
Chris Kelnar, (Co-PI), University of Edinburgh, UK
M. A. Wahed, (Coinvestigator) Laboratory Sciences Division, ICDDR,B
William Cutting, MB, (Coinvestigator) University of Edinburgh, UK

**Budget:** US $ 76,949

**Funding Source:** USAID

**Proposed starting date:** Upon availability of funds

**Duration of Project:** One year

**Scientific Division:** This protocol has been approved by CSD

**Signature of Division Director:** Dec 31, 1995

**Abstract:**

Children with iron deficiency anemia have impaired growth that can be corrected by supplementation with iron. However, recently it has been shown that iron supplementation in *iron-replete* children also has an adverse effect on growth. If confirmed, this has major implications for iron fortification/ supplementation programs. Iron is also important to maintain normal intestinal mucosal function. In this study, we propose to determine the effect of oral iron on growth and intestinal permeability in both iron-replete and iron-deplete children.

Endocrine and bone metabolism markers of growth and intestinal permeability will be characterized in thirty iron deficient and thirty iron-replete children aged one to five years before and after iron supplementation. Weight, height, and body composition will also be measured monthly and the results correlated with the biochemical markers of growth. We expect that the results of the study will have meaningful policy implications for iron supplementation programs.

## HYPOTHESES

1. Iron deficiency anemia (IDA) is associated with abnormal intestinal integrity.
2. Abnormal linear growth in children with IDA is the result of abnormalities of the growth hormone/insulin-like growth factor 1 (IGF-1) axis.
3. Abnormal linear growth in children with IDA is the result of abnormal bone metabolism.
4. Iron supplementation of iron-replete children will result in abnormalities of the growth hormone/insulin-like growth factor 1 (IGF-1) axis.
5. Iron supplementation of iron-replete children will result in abnormal bone metabolism.

## OBJECTIVES

1. Determine intestinal integrity in children with IDA and iron-replete children before and after treatment with ferrous sulfate.
2. Determine the function of the growth hormone-IGF-1 axis in children with IDA and iron-replete children before and after treatment with ferrous sulfate.
3. Assess indicators of bone synthesis in children with IDA and iron-replete children before and after treatment with ferrous sulfate.

## BACKGROUND INFORMATION

Iron deficiency is the most common nutritional deficiency worldwide, affecting millions of people.[1] The consequences of iron deficiency anemia (IDA) are particularly severe in children and include abnormalities of immune function, increased risk of infections, and deficits of cognition and motor function in infants which are potentially irreversible.[2,3,4,5] Children with IDA also have a decreased growth rate which can be corrected by supplementation with oral iron.[6,7,8] While the evidence for an association of iron deficiency with poor growth is compelling, recent evidence suggests that iron supplementation of iron-replete children might also have an adverse effect on growth. In a study by Idjradinata et al., iron-replete children supplemented with 3 mg/kg of ferrous sulphate daily exhibited poorer weight gain compared to the placebo supplemented group.[9] *Previously, an assumption of iron fortification programs has been that excess iron among people replete iron stores is inconsequential. If an adverse effect is confirmed however, this would have major implications for iron fortification programs.*

Demonstrating a causal role for iron deficiency in growth abnormalities within the context of clinical or survey studies is difficult however, since iron deficiency is often a comorbid condition with one or more environmental abnormalities such as protein-energy malnutrition, zinc

deficiency, and frequent or severe infections which themselves are established causes of growth failure.[10,11,12] However, iron is known to be important for the differentiation and maintenance of normal intestinal mucosal function.[13] In infants and young animals, chronic iron deficiency is associated with depressed disaccharidase activities in the jejunum which results in carbohydrate malabsorption.[14,15,16] Treatment with iron restores normal enzyme activity. Mechanisms at the intestinal level that explain the adverse effect of iron deficiency on growth as well as morbidity and mortality have not yet been demonstrated. Speculation might focus on two general areas; (1) changes at the level of mucosal surfaces that alter nutrient absorption or cell surface-pathogen interaction, or (2) a possible adverse effect on the host immune response that might result in an increased burden of infection. Of note, abnormalities at these two levels would not be mutually exclusive.

Differential sugar tests have been recommended as the most appropriate non-invasive means of investigating small intestine permeability.[17,18] This test is based on the simultaneous administration of two probe molecules, a monosaccharide and a disaccharide, and subsequent estimation of the urinary recovery of each molecule. In disease of the proximal small intestine the recovery of the monosaccharide is reduced, perhaps due to a reduction in absorption surface area, while that of the intact disaccharide is increased. The result is expressed as a ratio. A significant advantage of the differential sugar permeability test is that the effect of extraneous variables that influences a single test sugar such as xylose can be overcome by expressing absorption as a ratio of absorption of the two markers.[19,20] Variables such as adequacy of ingestion, gastric emptying rate, intestinal transit time, renal clearance, and completeness of urine collection do not alter the absorption since they affect both markers equally.[22] Abnormal permeability is also associated with diarrheal disease and with mucosal damage, thereby providing a reliable and useful index of mucosal integrity.[20,21,22]

Growth hormone (GH) is the most abundant pituitary hormone and a major promoter of anabolism. Growth hormone secretion occurs predominantly in peaks overnight during deep sleep. Early morning urinary growth hormone can be measured to assess overall growth hormone output from the pituitary. IGF-1 exerts a direct effect on somatic growth, and is believed to be the primary mediator of the growth promoting effects of GH. IGF-1 is synthesized in the liver and by target tissues such as muscle and cartilage. The two predominant regulators of IGF-1 are GH and nutritional status. IGF binding protein 3 (IGFBP3) is one of three identified IGF1 binding proteins, and acts as a reservoir of IGF1 in the circulation. Its concentration may be a better measure of IGF1 status than the IGF1 concentration itself.

Osteoclastic activity resorbs bone and occurs in association with osteoblastic bone synthesis during active bone turnover. Pyridinoline (PYD) and deoxypyridinoline (DPD), the amino-acid cross-links between the collagen fibrils in bone and cartilage, are released during resorption of bone and subsequently excreted unchanged in urine.[23] The total urinary pool of PYD and DPD is almost entirely derived from bone with only minimal amounts from tendons, ligaments and aorta, and none from dietary intake.[24] Their excretion exhibits a diurnal pattern, but with little change during the morning, and measurement of their concentration expressed as a ratio to creatinine is therefore an accurate assessment of excretion (personal communication, B Golden).

During the synthesis of type I and type III collagen, large soluble propeptides are released into the circulation and these can be assayed by radioimmunoassay. The amino terminal propeptide of type III collagen (PIIINP) and the carboxy terminal propeptide of type I collagen (PCIP) have been shown to reflect growth rates.[25,26,27] Type I collagen is found in bone and soft connective tissues and type III collagen in soft tissues alone, so that linear growth rate more closely correlates with PCIP than with PIIINP.[23]

Height increments are such that measurements at least 8 weeks apart are required before height velocity can be accurately assessed.[28] Portable knemometry by experienced operators permits far more accurate measurement of the heel-knee length, enabling meaningful length velocity assessment over shorter time periods.[29]

## METHODS

1. Study Population:

Sixty children, one to five years of age and without evidence of acute malnutrition as defined by weight-for-height >80th %ile or MUAC >13.5 cm will be enrolled. **Group I**, IDA (hemoglobin <10.5 g/dL and serum ferritin <12 μg/L); **Group II**, iron-replete children (hemoglobin >12 g/dL and serum ferritin >20 μg/L). All children will be studied before and after iron supplementation. Children will also be dewormed prior to testing and the intervention.

To enroll thirty iron-deplete and thirty iron-replete children, about 200 children need to be screened by fingerstick specimen (≈0.5 ml blood) for serum hemoglobin and serum ferritin levels. Written consent will be taken from the parents of these children and parents will be given compensation for the day's wage loss. The children will be recruited form Nandipara, a semiurban community at the periphery of Dhaka.

2. Biochemical Assessment:

- *Iron Status*

Hemoglobin (Hb) will be quantitated by spectrophotometry after conversion of Hb to cyanmethemoglobin.[30] Standards provided by WHO will be used as internal controls for Hb assessment. Serum ferritin will be measured by solid phase enzyme immunoassay.[31]

- *Endocrinologic Assessment*

Early morning GH excretion will be measured by radioimmunoassay (RIA).[32] Serum IGF1 and IGFBP3 concentrations will also be measured by RIA.[33]

• *Assessment of Bone Metabolism*

PYD and DPD will be measured by reverse-phase high-pressure liquid chromatography and expressed as a ratio to urinary creatinine concentration ($U_{PYD\ or\ DPD}/P_{PYD\ or\ DPD}$ divided by $U_{Cr}/P_{Cr}$).[34,35] Urine will be collected at the same time of the morning on each occasion and creatinine concentration will be determined. PCIP and PIIINP will be measured in blood by radioimmunoassay.[36,37] Bone alkaline phosphatase concentration in blood will also be estimated by lectin affinity electrophoresis.

3. Intestinal Permeability Test (IPT):

Permeability will be assessed utilizing a differential sugar absorption test in which permeability to lactulose and mannitol are determined before and after iron treatment. A baseline urine is obtained and the child fasted for at least 2 hours prior to testing. The child is then given the test solution containing 1 gram of mannitol and 5 grams of lactulose in 20 milliliters of 1% chloroform water. Urine will be collected for the following five hours and preserved with one drop of 20% wt/vol chlorhexidine gluconate. Aliquots will be stored at -4$^0$C until testing. Urine lactulose and mannitol will be quantitated by an enzymatic method established in our laboratories.[38,39]

4. Assessment of Growth and Growth Velocity

Measurements will include weight, height, MUAC, TSF, bioelectric impedance assessment (BIA), and knemometry. Naked weights will be obtained to the nearest 10 g using an electronic digital scale (Seca model 770, Sweden) standardized with a one kg standard weight prior to each weighing. Recumbent length will be determined with a locally constructed instrument using the pressure technique in which a metal tape measure is extended between a footplate and head bar. The mean of two consecutive measurements to the nearest 0.5 cm will be recorded as the observed value. All measurements will subsequently be compared to the standards according to the National Center for Health Statistic data and the nutritional status assessed by Z score.[40,41] Percent of median equivalents of standard deviation units will be determined and categorized according to the system of Waterlow.[42] Body mass index (weight/height$^2$), triceps skinfold thickness, and mid-upper arm circumference will also be assessed. BMI results will be compared to the standardized curves of the National Health and Nutrition Examination study while skinfold thickness and midarm circumference compared to the standards of Karlberg, et al.[43,44] The mean of two consecutive measurements for all indices will be recorded as the observed value.

Reactance and resistance will be measured with a RJL whole body plethysmograph model BIA-101. Total body water (TBW) will be calculated according to the formula of Fjeld, et al for use in infants and young children: TBW (kg) = 0.48 + 0.68 Height$^2$ (cm)/Resistance (ohms); (SEE = 0.36, r = 0.98).[45]

## SCHEDULE OF TESTING

Children will be assessed at baseline and treated for three months with 3 mg/kg/day of ferrous sulphate. Testing (growth markers and intestinal permeability) will be repeated after four weeks. In addition, anthropometry, knemometry, and BIA will be measured monthly for four months beginning with the start of the iron treatment.

## SAMPLE SIZE CALCULATION AND STATISTICAL ANALYSIS

Data related to growth velocity using knemometer and changes in biochemical markers of growth are used to estimate the sample size. With a power of 80% at 5% level, the formula $n=7.9\ (SD_1^2 + SD_2^2)/d^2$ is used where $SD_1$ and $SD_2$ are the standard deviations in groups I and II respectively and d is the difference in the outcome measures.

Bisgaard [46] showed that the average growth of lower leg length in children to be 92 $\mu$m/day with a SD of 69 $\mu$m/day. Expecting a difference of 50 $\mu$m/day increase in lower leg length, N=30. Branca *et al*[47] showed a difference in PYD of 21 nmol/h/m$^2$ between malnourished and recovered children. Standard deviations of the two groups were 4.6 nmol/h/m$^2$ and 10.8 nmol/h/m2. Using these data in order to show a difference of 10 nmol/h/m$^2$ in change of PYD between groups, N=11. Branca *et al* showed a difference of 4.9 nmol/h/m$^2$ in DPD between malnourished and recovered children. Standard deviations of the two groups were 1.3 nmol/h/m$^2$ and 3.0 nmol/h/m2. Using these figures in order to show a difference of 2.5 nmol/h/m$^2$ in change of DPD, N=14.

In considering all of the above, the sample size should be 30 in each of two groups, or a total of 60 children.

Study and control group comparisons of continuous data will be assessed by the Mann-Whitney U test (rank-ordered statistics). Comparisons of data of both groups before and after treatment with ferrous sulfate will be assessed using the Wilcoxon ranked sums test. Group comparisons will also be made among and between the iron deficient group (baseline and after treatment) and the iron-replete group (after treatment) with baseline values of the iron-replete group.

## SIGNIFICANCE OF STUDY & DISSEMINATION PLAN:

Research findings from this study are expected to have meaningful implications for iron supplementation programs and to provide new information on the mechanisms of the iron-growth and iron-intestinal integrity interactions. This study proposes to assess the effects of iron supplementation on growth using newer and more precise biochemical markers than have previously been applied to the investigation of these interactions. The results of this study will be disseminated through presentation at scientific conferences and through publication in peer-reviewed journals.

## ETHICAL CONSIDERATIONS:

The proposal will be thoroughly reviewed and approved by the Ethical Review Committee of the Centre. Written consent will be obtained from the guardian of the children participating in the study. Confidentiality will be maintained regarding data collection and about the investigation results. The subjects will have access to the information and retain the right to refuse to participate in the study or to withdraw from the study at any time.

Approximately 0.5 ml of blood will be obtained by fingerstick during the screening process. After enrollment, there will be two scheduled tests periods (before and after iron supplementation) in which approximately 5 ml of blood will be obtained by antecubital venipuncture and all urine voided for a 5 hour period will be collected for the intestinal permeability test. During these testing periods, the children will be maintained in the metabolic ward of the ICDDR,B the night before and the morning of the testing and be under medical supervision throughout. The mothers of the subjects will be allowed to stay with their children during the testing periods. Apart from the known complications of blood drawing which are generally minor including temporary discomfort/pain, potential for bruising at the site of venipuncture, etc, participation in this study poses no substantial likely risks to the subjects. All subjects will potentially derive benefit from participation in the study through the identification and treatment of iron deficiency if present as detected by the tests programmed in the protocol.

**Work schdule**

| Month | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Staff requirement | X | X | | | | | | | | | | |
| Screening | | X | X | X | | | | | | | | |
| Enrollment | | | | | X | X | X | | | | | |
| Follow-up | | | | | | X | X | X | X | X | | |
| Laboratory analysis | | | X | X | X | X | X | X | X | X | | |
| Data entry | | | | | | | X | X | X | X | | |
| Data analysis | | | | | | | | | | X | X | |
| Reports/Publication | | | | | | | | | | | | X |

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| IRON AND GROWTH | | | |
|---|---|---|---|
| **1. Personnel** | | | US $ |
| *Investigators* | | | |
| George Fuchs, MD (PI) | 5% effort x one year | | nil |
| Dr. Aminul Islam (Co-PI) | 30% effort x one year | | 4,548 |
| Dr. Chris Kelner (Co-PI) | 5% effort x one year | | nil |
| MA Wahed (Co-investigator) | 5% effort x one year | | nil |
| Dr. William Cutting (Co-investigator | 5% effort x one year | | nil |
| *Other Staff* | | | |
| Research Physician (Fellow) | 100% effort x one year | | 2,100 |
| Health Assistants (3) (Fellow) | 100% effort x one year x 3 | | 5,940 |
| Health Workers (3) | 100% effort x one year x 3 | | 1,800 |
| Statistician N/A | 10% effort x one year | | 900 |
| **Subtotal** | | | **10,740** |
| **2. Supplies** | | | |
| *Laboratory Supplies* | | | |
| Anemia screening | $20 x 200 | | 4,000 |
| At ICDDR,B | $25 x 2 x 60 children | | 3,000 |
| At Edinburgh | $150 x 2 x 60 children | | 18,000 |
| Miscellaneous (cryovials, syringes, pipettes, etc.) | | | 3,000 |
| *Patient Costs* | | | |
| Ward costs for in-patient testing | $15 per d x 10 d x 60 patients | | 9,000 |
| Compensation for travel to hospital for testing | $10 per visit x 2 visits x 60 patients | | 1,200 |
| Medicines as required | $5 per patient x 60 patients | | 300 |
| *Supplies For Communication, Data Collection* | | | |
| Fax, telephone, photocopy, paper, etc. | | | 1,200 |
| Manuscript preparation | | | 300 |
| *Other* | | | |
| Knemometer | | | 5,000 |
| **Subtotal** | | | **45,000** |
| **. Travel** | | | |
| Presentation of results at international meeting (airfare, *per diem*). | | | 3,000 |
| **Subtotal** | | | **3,000** |
| **. Direct Costs** | | | **58,740** |
| **Indirect Costs (31%)** | | | **18,209** |
| **. Total Cost** | | | **$76,949** |

31/12/95

31/12/95

# CONSENT FORM

"Effect of iron supplementation on growth (ICDDR,B)"

The purpose of this study is to see the effect of iron supplementation on growth of your children. If you decide to allow your child to participate in this study, we will keep your child in our hospital for one day. During this stay, we will take weight, length and other measurements of growth. To see intestinal permeability, your child will be fed sugars (lactulose and mannitol) and their excretion in urine will be measured by collecting urine for 5 hours (using polyvenyl-urinary collection bag). About one tea-spoonful (5ml) of blood will be collected by venipuncture. Your child will be given iron supplementation for 3 months and weight, length and other measurements of growth will be repeated every month for four months. Intestinal permeability test will be repeated after one month of iron supplementation, when another one tea-spoonful (5 ml) of blood will be collected by venipuncture.

The study involves no likely potential risk to your child. There will be minor, temporary discomfort due to blood drawing. For the daily wage loss you will be offered a compensation package of Tk. 100.00 for each visit for the purpose of study.

If you agree, we will include your child in the study. If you do not agree, then your child will get all the normal treatment from this hospital. If you agree now, but later change your mind, then you can remove your child from the study immediately. If you are happy for your child to be included in the study, please give your consent by signing this form (or putting left thumb impression). Thank you for your cooperation.

Guardian's signature/Left thumb impression: _____

Investigators signature: _____

Date: _____

# CONSENT FORM

"Screening for Iron Status (ICDDR,B)"

The purpose of this study is to see if your child is deficient in iron. Depending on the results of blood test, we may call you to allow your child to participate in a study to see the effect of iron supplementation on growth.

If you allow your child to participate in the study, we will take about half ml of blood by finger prick. There is no potential risk due to blood drawing, apart from minor temporary discomfort of blood drawing. If your child is found to be iron-depleted, he/she will be supplemented with iron.

You may refuse to participate in the study or you may, at anytime, withdraw your consent during the course of the study. In either case the treatment for you or for your child will not be affected by your decision.

If you decide to allow your child to participate in the study, please put your signature/left thumb impression below. Thank you for your cooperation.

Guardian's signature/Left thumb impression: _____

Investigators signature: _____

Date: _____

Reviewer 1



Title: The effect of iron status on intestinal integrity and growth.

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

| | Rank Score | | |
|---|---|---|---|
| | High | Medium | Low |
| Quality of Project | | | |
| Adequacy of Project Design | X | | |
| Suitability of Methodology | X | X | |
| Feasibility within time period | X | | |
| ~~Appropriateness of budget~~ | X | | |
| Potential value of field of knowledge | - | - | - |
| | X | | |

CONCLUSIONS

I support the application:

a) without qualification [X]

b) with qualification

- on technical grounds [ ]
- on level of financial support [ ]

**Detailed Comments**

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

(Use additional pages if necessary)

Title: The effect of iron status on intestinal integrity and growth.

PI:

Reviewer: P.E. Cornwell, Ph.D.

I think that this project has the potential to answer a very important research question concerning the safety of iron supplementation.

My only concern is that the number of research subjects seems low. Perhaps this aspect of the proposed project could be followed more closely by a biostatistician.

Just a housekeeping comment: On page 4, references 30 and 31 are not appropriate to the discussion of quantitation of hemoglobin. Also, reference 36 is missing the year of publication. Should reference 40 and 41 be reversed? (at least in reference to their publication dates.)

Reviewer No. 2



Title: The effect of iron status on intestinal integrity and growth.

Dr Fuchs

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

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

CONCLUSIONS

I support the application:

a) without qualification ✓

b) with qualification

- on technical grounds
- on level of financial support

I do not support the application

**Detailed Comments**

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

(Use additional pages if necessary)

Title: The Effect of Iron Status on Intestinal Integrity & Growth

PI:

Reviewer: Professor Quazi Salamatullah

Nutritional anemia is one of the major nutritional problems among children and pregnant women in Bangladesh. Different dietary surveys show that there is enough iron intake among the population, still lot of people are anemic. This may be due to overestimation of dietary iron intake ( mostly measured on raw food basis, not on the basis of as eaten food ) or due to low vit A / vitamin C intake or abnormalities of intestinal integrity.

The present study is designed to observe 1) the intestinal integrety in children,2) the function of growth hormone IGF-1 axix in children,and3) the indicators of bone synthesis in children with iron deficiency anemia and iron-replete children before and afte: treatment with ferrous sulfate.

The study seems to be original, it is feasible, and expected to provide new knowledge.However,I have following comments/suggestions:
1) The children will be treated for 3 months. In the literature review it is mentioned that " height increments are such that measurements at least 8 weeks apart are required before height velocity can be accurately assessed". I think the effect of ferrous sulfate supplementation should be observed for 4 months.
2) Total 30 children will be enrolled which will be divided into two groups. In long run some children may be dropped. It is better to increase the subject number.
3) There is no mention about the study site, subjects from low or middle or high socioeconomic group ?
4) In the methodology it is mentioned that PYD and DPD will be measured and the result will be expressed as a ratio to creatinine concentration. How urinary creatinine will be measured is not mentioned or without measuring urinary creatinine how the ratio will be expressed is not clearly mentioned?

**IRON SUPPLEMENTATION, GROWTH, INTESTINAL PERMEABILITY PROJECT:RESPONSE TO REVIEWER**

**Reviewer #1**

a) Both reviewers #1 and #2 expressed concern that the subject number might be too few. The sample size of 15 subjects in each of the two groups was originally intended to serve as a pilot. However, we have revised our estimates of sample size requirements as described in the proposal and have arrived at a figure of 30 subjects for each of the two groups, which is double the sample size of the original proposal.

b) The references have been corrected.

**Reviewer #2**

a) We will serially monitor growth (anthropometry, including knemometry) for four months as suggested.

b) The sample size will be increased as described above.

c) Subject recruitment will be in the community of Nandipara, a low socioeconomic community.

d) PYD and DPD will be measured by reverse-phase high-pressure liquid chromatography and expressed as a ratio to urinary creatinine concentration. The standard formula that exists for this type of calculation will be used i.e., $U_{PYD\ or\ DPD}/P_{PYD\ or\ DPD}$ divided by $U_{Cr}/P_{Cr}$. This has been included in the proposal. A description of the details of the methodology for the quantification of creatinine in urine and plasma has not been included in the proposal because this is a routine, noninvestigational clinical laboratory test.