Attachment 1.

Date 02.03.94

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. Iqbal Kabir

Application No. 94-004 (Revised)

Title of Study Vegetable protein source for refeeding malnourished children during recovery from shigellosis.

Trainee Investigator (if any)

Supporting Agency (if Non-ICDDR,B)

Project status:
( ) 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)
(b) 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)
NA 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.

Iqbal Kabir
Principal Investigator

Trainee

RESEARCH PROTOCOL

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TITLE: VEGETABLE PROTEIN SOURCES FOR REFEEDING MALNOURISHED CHILDREN DURING RECOVERY FROM SHIGELLOSIS

Starting date: April 1, 1994

Completion date: 2 years after start

Total budget requested: US $ 238,771

Funding source: A small partly by International Atomic Energy Agency (IAEA), Vienna, Austria & other sources to be explored

Programme Head:

Dr. Dilip Mahalanabis
Associate Director, CSD

Abstract summary

Shigellosis is a major cause of childhood mortality in developing countries. Approximately 300 million children suffer from this disease and, about 600,000 die annually due to shigellosis. A substantial proportion of those children who survive develop secondary protein-energy malnutrition (PEM). Refeeding children with secondary PEM requires increases in dietary energy, protein, and micronutrients. Previous studies at ICDDR,B have shown accelerated catch-up growth with a high-protein (animal) diet during recovery from shigellosis. Since the sources of dietary protein we used are too costly in most

communities where PEM is common, we propose a low-cost plant-protein based dietary supplementation to accelerate catch-up growth in malnourished children with shigellosis during recovery.

The study will be conducted in 90 Bangladeshi children aged 2-5 yr, who will be hospitalized in a metabolic refeeding ward for 3 weeks. The relative efficacy of a standard reference 7% protein diet recommended by WHO/FAO for refeeding undernourished children will be compared with 2 nutrient dense formulas, one based on animal protein to provide 15% of energy as protein and the other based on plant protein to provide 15% of energy as protein with micronutrients supplemented to the later diet to match the intake in the animal protein group. The randomly assigned diets will be provided for the 21-day study. Dietary tolerance, diarrhoeal severity and duration, signs of infection will all be monitored. Serial measurements will be made of body composition using anthropometry and bioelectrical impedance, and of IGF-1 and IGF-binding proteins. Protein anabolism will be measured early in the recovery and again after 21 days of dietary therapy. The study will thus test the hypothesis that the 15% protein diet, whether based on animal or vegetable protein, stimulates anabolic drive, accelerates catch-up growth in both weight and length, and increases concentrations of IGF-1 and IGF-binding protein more than reference vegetable-based diet containing 7% of energy as protein. The principal outcome variables are : 1) body compositional change as assessed by standard anthropometric methods and by bioelectrical impedance; 2) concentrations of IGF-1 and IGF-binding proteins; 3) protein anabolism as assessed by increased ratios of protein synthesis to protein breakdown. During the 3-week recovery period, anthropometric measurements will be made twice weekly, resting energy expenditure and protein cycling will be measured twice on day 1 and at the end of the study.

The studies of protein turn-over will be done using the stable isotope tracer of protein metabolism, ^{13}C-leucine and $^{2}H_3$-leucine, which can be administered safely to children because of the fact that it is neither radio-active nor do they alter normal metabolism. The protocol includes some preliminary studies to establish the most reliable and practical methods of isotope administration and sample collection. Samples of urine, plasma and breath for tracer will be analyzed by mass spectrometry and isotope ratio mass spectrometry in the laboratory of Dr. David Halliday, the collaborator in this study, who has more than twenty years of experience in the investigation of protein metabolism and its relationship to growth in children.

The results of this study will determine whether the anabolic effects documented during refeeding with large amount of dietary protein from animal source are critical or equivalent rates of growth are achievable with a more economical plant-protein based diet.

Aims of the project

[a] **General aim:** Our previous studies at ICDDR,B have shown a rapid catch-up growth of malnourished children fed an animal protein-based diet during recovery from shigellosis. This study will be conducted to evaluate whether a low-cost, plant-protein based diet has similar efficacy in enhancing catch-up growth of malnourished children during convalescence from shigellosis. This plant-protein based (legume-cereal) is commonly available in most developing countries and consumed daily.

[b] **Specific aims:**

1. To evaluate the effects of a legume-based protein diet to promote catch-up growth of malnourished children during recovery from shigellosis.
2. To determine the role of insulin-like growth factors (IGF-1) and IGF binding proteins(IGFBPs) as indices for catch-up growth in malnourished children.
3. To determine the changes in body composition of malnourished children after feeding with a legume-based diet during recovery from shigellosis.
4. To determine the effects of feeding a legume-based diet on further growth and morbidity during a 3-month follow-up at home.
5. To determine the protein turn-over in malnourished children after refeeding the study diet by using stable isotope techniques.

[c] **Significance and International Nutrition Implication:**

Protein-energy malnutrition remains a major health problem in most developing countries. The magnitude of this problem is more obvious in underprivileged community in least developed countries like Bangladesh. In a recent survey by Bangladesh Bureau of Statistics (1991), about 35% of under-five children were severely stunted (below -3 SD height-for-age). About 40% of children were both severely underweight and stunted. The causes of PEM, are perhaps, related to several factors including poverty,improper weaning practices, recurrent infections, and also inadequate diet during and after infection. Therefore, there is a urgent need to develop a designer food which can be supplemented to prevent growth faltering and enhance catch-up growth of children suffering from recurrent infections with secondary PEM.

The results of this study will help us to determine whether the anabolic effects documented during refeeding with large amounts of dietary protein from animal sources are critical or whether equivalent rates of catch-up growth are achievable when more economical vegetable-based proteins either as they are normally prepared or with the addition of micronutrients, are used instead. These will thus clarify whether the source of protein matters, as well as add to the body of knowledge concerning the optimal level of protein to provide in a mixed diet. Furthermore, the results will be useful to learning whether the acute responses in term of protein synthesis and breakdown, which can be measured with stable isotope tracers, predict anabolism classically measured by anthropometrics. This outcome is also important because if this is demonstrated, it will pave the way for a number of studies which can be designed to study precisely how dietary treatment can be tailored to stimulate the anabolic drive in children during recovery from PEM.

Ethical implication:

There is no ethical concern for this study. The children are expected to be benefited with the dietary intervention. The stable isotopes ^{13}C-leucine and $^{2}H_3$-leucine which can be administered safely to children because of the fact that it is neither radio-active nor do they alter normal metabolism. For assays of serum proteins and isotope study maximum 5 ml of blood will be required before and after the study.

Introduction:

Shigellosis as a global problem: Shigellosis remains a major cause of childhood morbidity and mortality in many developing countries. In a recent estimate of disease burden it has been found that nearly 300 million children suffer from shigellosis. Of these , nearly 6 million are considered to be severe and about 650,000 cases are expected to die annually (Lindberg AA, 1986). Most of these deaths are due to several life-threatening complications such as hemolytic-uremic syndrome (Butler, 1987), sepsis (Struelens, 1985), intestinal obstruction (Bennish, 1991), hypoglycemia (Bennish, 1990), and hyponatremia (Kabir, 1988).Nevertheless, most of these cases also have protein-energy malnutrition secondary to shigellosis. The risk of death was increased 4 times in sepsis with third degree malnutrition, and 14 times after discharge from the hospital in those patients having dysentery with severe PEM, as compared to children with uncomplicated shigellosis.

In the late nineteenth and twentieth centuries, numerous outbreaks were recorded with *Shigella* causing high morbidity and mortality in most continents of the world (Kostrzewsky 1968). In Europe and North America, shigellosis was an important problem during the first quarter of this century. Improvements of living conditions such as housing, sanitation, and nutrition have reduced the spread of this organisms. However, in most underdeveloped countries, the conditions that favor endemicity of shigellosis are still present, resulting in continuous transmission of the disease with periodic occurrence of outbreaks and epidemics.

During 1969-1970, a vast and unusually severe epidemic of shigellosis occurred in Central America causing nearly 30,000 deaths (Mata, Gangarosa 1970). During 1974, an outbreak of dysentery caused by *S. dysenteriae* type 1 occurred in a coral island in the Bay of Bengal with a mortality rate of 2% affecting mainly the very young and elderly (Rahman 1974). The case fatality rate of 6.4% among those with a history of a clinical shigellosis was comparable with the rate of 7.4% reported from Guatemala (Gangarosa 1970). In contrast, the case fatality in El Salvador was only 2%. In Bangladesh the case fatality rate amongst infants and children was 41% (Rahman 1974) , but there were no deaths in those 10-50 years old, and patients over 50 years age had an increased death rate.

Diarrhoea-malnutrition interaction:

Prospective studies of growth and morbidity have identified certain infections as particularly important as causes of poor growth. Among these, diarrhoea, respiratory tract infections, and malaria are most common. The impact of infection on growth may vary according to the previous nutritional status of the child, the availability of food, cultural beliefs, and access to health facilities. Rowland et al (1977) have shown a marked negative effect of diarrhoea and malaria on weight gain in an underprivileged community in rural Gambia. Diarrhoea also caused a reduction in height gain.

Diarrhoea has less impact on the nutritional status of younger infants than that of older children. Children receiving solid foods in Guatemala (Mata et al 1977), Gambia (Tomkins, 1983), and Bangladesh (Hoyal et al 1980; Molla et al. 1983), have shown reduced intake of solid foods during diarrhoea. Subsequently, another study in Bangladesh showed no significant decrease in

solid food intake in children (Brown et al 1985).

The impact of diarrhoeal diseases on intestinal absorption of nutrients has been reviewed by Tomkins AM (1981). During acute phase of diarrhoea a high level of absorption of macronutrients is maintained which further improves during the recovery phase (Molla et al. 1983). However, there may be severe malabsorption with endogenous protein loss that occurs in diseases such as protein-losing enteropathy, and shigellosis complicating post-measles diarrhoea (Sarker et al. 1985).

Nutritional Consequences of Shigellosis:

Children in developing countries show slower growth rates than children in developed countries. Many are stunted when they reach adulthood. A large part of this growth retardation is caused by malnutrition due to decreased food intake during infectious diseases. Scrimshaw (1977) has attributed the decreased intake of calories during infection to a combination of anorexia, fever, withdrawal of solid foods by parents, impaired intestinal absorption, and direct loss of nutrients in the stool. The effects of diarrhoeal diseases on the growth of Bangladeshi children has been studied prospectively by Black et al (1984) who have shown that shigellosis had a significantly negative effects on linear growth and diarrhoea due to *E. coli* had negative effect on ponderal growth. In another study it has been found that stunting was significantly associated with dysenteric illnesses (Henry et al 1985). Black and his colleagues also have shown that shigellosis had a longer duration than other types of diarrhoea with 16% of episodes lasting for more than 20 days. Study by Kabir et al (1985) has shown that patients with shigellosis passed bulky voluminous stools suggestive of intestinal dysfunction for several days even after clinical recovery. Roy et al (1983) have shown an excess mortality in malnourished children after discharge from the hospital, and 60% of those children had dysentery. These findings indicate that a comprehensive nutritional rehabilitation program is needed to reduce malnutrition secondary to shigellosis and to promote catch-up growth during the recovery period.

Protein-losing enteropathy in shigellosis:

The magnitude of protein loss in shigellosis is much higher than in other acute watery diarrhoeas and a patient can lose up to 500 ml serum in a day during severe shigellosis (Rahaman MM 1983, Black 1991, Bennish 1992). This leaking of serum protein may persist even during the recovery period, especially in children with measles (Sarker 1985). In children of developing countries who are already in a marginal nutritional status, this continuous serum protein loss is detrimental. In Bangladeshi children with severe shigellosis serum protein concentrations were found to be nearly half of the normal value (Butler 1987). In severe malnutrition, depressed serum albumin concentrations are instrumental in lowering plasma oncotic pressure and allowing the edema of kwashiorkor to develop (Coward 1979).

Dietary Management of Diarrhoeal Diseases during Acute and Convalescence Stages:

Optimal feeding practices during acute diarrhoea are still controversial; this was reviewed by Brown et al (1984). The theoretical advantages of delayed feeding include the avoidance of increased fluid loss, acidosis, and mucosal

injury caused by certain foods. The advantages of continued feeding during diarrhoea are to prevent weight loss and protein deficits, to maintain and repair injured mucosa, and to sustain the benefit of breast feeding. The World Health Organization has recommended the continuation of breast feeding; the weaning diet should be continued as previously except that cow's milk and formula milk should be diluted (Brown KH 1984). These recommendations apply mainly to watery diarrhoeas that affect the small intestine. Less attention , however, has been given to shigellosis and other invasive diarrhoeas affecting the colon (Butler T, 1986). Recently, Kabir et al (Am J Clin Nutr 1993, Ped Res 1992) has demonstrated that catch-up growth could be accelerated during recovery from PEM secondary to shigellosis by feeding a diet with substantially more high-quality protein than is commonly recommended. The linear growth rates in that study in the control group of children fed the standard-protein diet was almost that of American children based on NCHS standard, whereas the linear growth rates in children fed the animal protein based high-protein diet exceeded the expected growth rate.

Increases in linear growth have also been observed in Bundi children of New Guinea who were fed a high-protein diet (Malcolm 1974). It has been suggested that height gain in Bundi children may have been related to the quantity of supplementary protein, whereas additional energy derived from fat caused an increase in body weight only in the control group. This observation suggests that during rapid weight gain on a high-energy formula, there is a tendency towards more deposition of fat tissue with a limited deposition of lean tissue, which indeed reflects an inadequate intake of protein either in quality or quantity (Jackson AA 1991). In another study in Colombia, diarrhoea was negatively associated with body length among children in the unsupplemented group; however, diarrhoea had no effect on length in those who were supplemented with a high-protein diet (protein-energy ratio 14-15%) (Lutter CK, 1989). Similar findings have also been observed in Guatemala and Brazil (Lutter 1992).

Role of Insulin-like growth factors (IGF) and insulin-like growth factor binding proteins(IGFBP) in regulating growth rate

Insulin-like growth factors IGF-I and IGF-II are a family of small peptides that are abundant in plasma and bind to larger proteins (IGF binding proteins). The IGFs resemble pro-insulin in amino acid sequence and have anabolic insulin-like actions on fat and muscle. Their growth promoting effects include enhancement of cell multiplication and stimulation of cartilage proliferation. Plasma concentrations of IGF-1 have been shown to fall by 60-70% in normal volunteers fasted for 5 days and return to normal levels within 8 days of refeeding (Isley WL 1983). This fluctuation which correlates well with changes in nitrogen balance (r=0.90), are regulated by the composition of the refeeding diet (Isley 1984). Because the IGF levels change rapidly with dietary manipulation in normal individuals, their measurement may be useful in predicting short-term changes in nutritional status. IGF-II values also declines in malnourished children, but are not subject to the rapid changes seen with IGF-I. IGFBP-1 rises with food restriction, whereas IGFBP-3 and IGFBP-2 decline.

In a recent study Kabir et al (1992) showed that successful dietary treatment of children recovering from shigellosis was associated with increased levels of growth factors IGF-I and IGF-binding proteins(IGFBP-2 and

IGFBP-3). These findings suggest that the mechanism by which anabolic effects they produced occurred secondary to the production of IGF-I and IGFBP which would increase protein synthesis and net protein anabolism. In a further study after refeeding malnourished children recovering from shigellosis (Pucilowska, Davenport, Kabir et al 1993) serum concentrations of IGF-I increased significantly more in children who were fed a high-protein diet (PER 15%) compared to those fed a normal protein diet (PER 7.5%). IGF-II increased more than 2 folds on each diet reaching the control values (well nourished American children of same age group). IGFBP-2 concentrations were twice of those in control and normalized after refeeding in the high-protein group. IGFBP-3 levels before refeeding were low and returned to normal on each diet. IGFBP-3 proteolytic activity in serum was initially increased and declined on the high-protein diet. These results show that protein content in the diet differently affects IGFs and IGFBPs in the young malnourished children with infection. IGF-I and IGFBP-2 seem to be particularly sensitive to dietary protein alterations. Therefore, measurements of these indices may be helpful to monitor the recovery and catch-up growth of malnourished children during dietary supplementation. Our findings are important because they imply that dietary intervention can be applied to stimulate the anabolic drive and thereby speed recovery from PEM.

Rational for using legume-based high-protein diet

Since the sources of dietary protein we previously used were mainly derived from animal proteins, which indeed are too costly for most communities in the developing countries where PEM is common, the next step is to determine whether more economical sources of protein can be substituted while maintaining the anabolic effects of high-quality protein.

For this purpose a legume-cereal based diet, khichuri has been proposed for refeeding children with shigellosis during convalescence. Rice and dal (red gram/lentil) are the major sources of energy and protein and are widely available throughout the developing countries. Although rice is deficient in lysine and dal is deficient in methionine, combining these two (khichuri) will make a balanced diet with all the essential amino-acids and will likely meet the daily requirements.

In a recent study Nigerian malnourished children who were fed a vegetable protein rehabilitation diet showed a satisfactory recovery in growth (Smith 1989). Increases in concentrations of plasma IGF-I were comparable with those found in an earlier study with a milk-based diet fed to malnourished Jamaican children (Payne-Robinson 1986). In both the Jamaican children fed a milk-based diet and Nigerian children fed a vegetable protein-based diet, the increases in insulin and plasma IGF-I concentrations were accompanied by significant increases in body weights.

As this legume-protein based diet might be low in zinc, we postulate that lean tissue repletion could be limited by the dietary zinc supply (Golden & Golden 1981). Zinc is essential for the normal function of several hormones involved in lean tissue as well as DNA synthesis and, in a less understood way, protein synthesis (Hambridge 1986). Giugliano & Millward (1987) have shown that zinc deficiency specifically inhibited muscle protein synthesis in rats. It has been also shown that in recovering malnourished children, plasma zinc concentrations fell to low levels during recovery, specially on a high-

energy, soy-based formula (Golden & Golden 1981a).

It is expected that other micronutrients such as folic acid, vitamin A, iron, calcium and phosphorous content will also be different in the three diets and could limit the catch-up growth. Therefore, we propose to supplement micronutrients zinc, folic acid, vitamin A, calcium and iron to match the intake in the animal protein group. This will be given in a daily oral dose as a vitamin mixture.

The other important factors are phytate content of the vegetable protein based diets. To minimize the phytate we shall use polished rice and ferment the legume before cooking. This fermentation process has been shown to remove 80% of the phytate content and improve digestibility of cereals and legumes.

Methods:

Study design. This study will be conducted in pre-school Bangladeshi children who will be hospitalized in a metabolic-refeeding ward during the 3-week course of study. Subjects will be randomly assigned to one of three dietary groups. The protein turnover will be studied twice, once during the acute-phase and another after a 3-week of nutritional intervention. Thus each subject will serve as his/her own control.

Subjects: Children aged 24-60 months whose parent or guardian consent to the child remaining in the hospital for 3 weeks will be admitted if they meet the following criteria listed below. At least one patient per week can be admitted during the 2-year period of the study.

Inclusion criteria

a. History of blood-mucoid stool for less than 5 days
b. Stool culture positive for *Shigella spp*
c. Length-for-age < -3.0 Z score by NCHS
d. Weight-for-length < -1.5 Z score by NCHS
e. No frank kwashiorkor
f. No complicating illnesses such as pneumonia, tuberculosis, septicemia
g. No hemolytic-uremic syndrome
h. Parental consent

Sample size calculation: From the NCHS standards and from our previous study (Kabir AJCN 1993), in children aged between 18 and 60 months the mean monthly increment in height is 6.9 mm with a variance of 3.4 mm. To achieve an alpha error of 0.05 and a power of 80% for a worthwhile difference between the diets of 2 mm per month in height, the required sample size is 28 in each group. Calculating for dropouts the total sample size is 90 patients; 30 in each group. However, to determine the protein turn-over using deuterated and ^{13}C-leucine, only 10 patients in each group will be studied.

Initial examination:

Clinical: On admission a clinical history and physical examination will be completed initially to assure that each patient fulfills the entry criteria for the study. The information obtained on admission will also be used to compare the two treatment groups, to assess their initial similarity. The

history and physical examination will contain the following information.

a. Identification of patient by name, date of birth, date of admission, age, gender, address, study number
b. Description and duration of symptoms prior to admission, characteristics of stool, presence of vomiting, abdominal pain, straining, fever, and change of appetite
c. Diet during the previous 24 hours
d. Treatment provided prior to admission, including oral rehydration solution and medications
e. Physical examination for degree of dehydration, nutritional status, and any abnormal physical findings
f. Weight, height, mid-arm circumference, triceps, biceps, suprailiac, and subscapular skinfolds and head circumference will be recorded.

Laboratory: On admission analysis of stool and blood culture will be performed to assure that each patient fulfill the entry criteria:

a. Stool microscopic examination for faecal leucocytes, red blood cells count stool culture and sensitivity
b. Hematocrit and complete blood count
c. Venous blood will be analysed for serum proteins, serum zinc, IGF-I, IGF-II and IGFBPs

These measurements will be repeated as needed in any patient who manifests clinical signs of diarrhoea and infection.

Concentration of serum proteins will be determined by standard techniques at ICDDR,B. Serum zinc will be assayed by atomic absorption spectrometry and IGF-1, IGF-II and IGFBP-1, IGFBP-2 and IGFBP-3 will be determined at Dr. L.E. Underwood's laboratory at the University of North Carolina at Chapel Hill, using specific radioimmunoassays (Kabir et al Ped Res 1993).

Randomization: After inclusion in the study the children will be randomly assigned to either a animal-based High-protein (15% of energy as animal protein) diet, or to a plant protein-based high-protein (15% of energy as grain /legume-based) diet or the reference standard protein diet (7% of energy, derived from plant protein). Random assignment to study groups will be made using the sequential numbers from a random number table. The sequential number with the diet assigned will be kept in a sealed envelope and will be opened just before the study begins; thus will allocate the dietary regime to each patients.

Anthropometry; Body weight will be obtained on admission and daily during a scheduled time (9 A.M) on a SECA scale with a precision of 10 g , the heights will be measured on a length board on admission and every 3-d until the study is completed, and there after every 15-d during follow-up. At least 3 measurements will be obtained by trained health assistants. MUAC, SKFT will be measured on admission and after 21-d. Body composition of the children will be measured with a bioelectrical impedance analyzer (BIA 101A RJL Sys, Detroit MI) and energy expenditure by indirect calorimetry.

Treatment Protocol: All children will be treated with an appropriate antimicrobial (pivmecillinam) for 5 days. After the clinical cure which will be determined by absence of fever, straining during defecation, blood and mucus in stool and reduction of stool frequency to less than 4 stools per day. The relative efficacy of a standard diet recommended by the FAO/WHO for refeeding PEM will be compared with two nutrient dense formulas, one based on animal protein to provide 15% energy as protein and other based on a plant-protein based diet (Khichuri) to provide 15% of energy as protein with trace elements supplemented to match intake in the animal protein group. The dietary treatments will be assigned randomly and will be provided during the 21-day study period. The diet will be provided ad libitum up to a maximum of 150 kcal/kg.d. divided in six feedings. A micronutrient mixture containing zinc, vitamin A, calcium, iron, folic acid and other vitamins will be given orally as a single daily dosage as per RDA (recommended daily allowance) for 21 days.

Protein kinetic studies: Protein kinetic studies will be performed on a subset of each dietary group before and after the 21-day dietary therapy. (I-^{13}C) leucine and $^{2}H_3$leucine (1mg/kg/h) will be infused by intravenous and nasogastric routes, for 4 to 6 hours, respectively. The intravenous infusions will be prefaced by priming doses of I-^{13}C-leucine (1 mg/kg) and ^{13}C-bicarbonate (0.18 mg/kg) (Matthew Am J Phys 1980). The nasogastric infusions will be by tube into the small intestine (aspirate pH checked). Protein kinetic studies will be conducted following an 8-hour overnight fast, or in the face of small repetitive oral feeds (every 30 minutes) to mimic energy expenditure at the resting metabolic rate. The resting energy expenditure (REE) will be measured in the "postabsorptive" state which, for practical reasons, may not be longer than 6h after a feeding. The REE will be measured for ≥ 30 minutes, during which the child is quiet and in a thermal neutral and quiet environment on a day immediately prior to the infusion study.

Calculation of protein kinetics i.e.., the rate of protein synthesis, break down and oxidation, will be based on measurements of enrichment of labelled leucine and its ketoacids (ketoisocaproate) (Thompson Eur J Clin Invest 1989) in the plasma and in the urine which will be performed by gas chromatography-mass spectrometry in the laboratory of Dr. David Halliday. Plasma and expired air samples will be collected before the infusion label (baseline) and at 30 minute intervals over the last 2 hours of the infusions. The intravenous infusions of I-^{13}C-leucine will provide flux (Q), oxidation (O) and intake (I) data directly and the rates of whole body protein synthesis (S) and breakdown (B) indirectly, according to the equation: Q = S + O= B + I. The nasogastric infusions will provide information on the splanchnic utilization of leucine (Matthew Am J Phys 1993). Importance is attached to the studies of fed state, i.e., during the provision of amino acids as substrate, as the dietary amino acids will maximize the potential level of net protein synthesis (pre- and post-dietary therapy) which might be masked were the studies conducted at fasted state.

Treatment failure: Children with recurrence of dehydration ≥ 5% and/or abnormal values of serum electrolytes or urinary specific gravity will be rehydrated orally and the diet will be suspended for the remainder of the day. At the end of this interruption, the study diet that was assigned initially will be reintroduced *ad libitum* at full concentration until the specific upper

limits on volume or energy intake is achieved. Elevated blood urea nitrogen will also be a criterion for interrupting the study, since this may suggests excess nitrogen intake. This is unlikely ,however, since the diets in the previous protocols with comparable levels of dietary protein were well tolerated (Kabir 1992 & Kabir 1993).

Body composition Study: To monitor the impact of supplementing the different study diets on body composition, total body water will be determined with stable isotope; deuterium oxide before the study begun and at the end of 21-d study period. The measurements of tracer (D_2O) will be done at Dr. Halliday's laboratory in UK by mass spectrometry using standard technique. This assay will allow us to detect the changes in fat free mass of those supplemented children and give us a better idea of nutritional status. Our previous study has found a strong relationship between height and body weight changes with fat free mass (Kabir et al AJCN 1993).

Home follow-up measurements: To test the possible beneficial effects of legume-based high-protein diet on subsequent growth and health, home visits will be made every week for a 3-month period. During follow-up body weight, height, MUAC, and SKFT will be measured. Disease morbidity such as diarrhoea, respiratory tract infections, skin infections and other morbidity will be recorded. A 24-h dietary recall will also be recorded during the home visits.

Data analyses: Increments of weights (g/kg.d) and heights (mm per day) will be calculated and compared among the groups as well as to NCHS standards for that age group. The means of increments of height and weight during each months period and at the end of study will be compared by Student's t-test. Analysis of variance(ANOVA) will be used to compare the three different dietary groups. NCHS package (NCHS-CDC) will be used to compare the Z-score value for anthropometry. Rates of diarrhoea, and other infections will be compared by Fishers Exact test, Chi-squire test.

Collaborative arrangements: This study will be carried out at the ICDDR,B in collaboration with Dr. Carla R. Fjeld at IAEA-CRP, Vienna, Austria, and Dr. Dave Halliday; Nutrition Research Group, Clinical Research Centre, Watford Road, Harrow, Middlesex Hai 3OO, UK, and Dr. LE Underwood, University of North Carolina at Chapel Hills, NC, USA (for measuring IGFs and IGF-BPs).

Last five years' publications of the Principal Investigator

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8. Rahman MM, Kabir I, Mahalanabis D, Malek MA. Decreased food intake in children with severe dysentery due *Shigella dysenteriae* 1 infection. Eur J Clin Nutr 1992;46:833-8.

9. Hussain MA, Kabir I, Albert MJ, Alam K, Kibriya AKMG, Alam AN. Campylobacter jejuni bacteremia in children with diarrhoea in Bangladesh: report of six cases. JDDR 1992;10:101-4.

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13. Kabir I, Speelman P, Islam A. Systemic allergic reaction and diarrhoea after pineapple ingestion. Trop Geo Med 1993;45:77-79.

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17. Kabir I, Malek MA, Rahman MM, khaled MA, Mahalanabis D. Changes in body composition of malnourished children after a dietary supplementation as measured by bioelectrical impedance. Am J Clin Nutr 1994;59:5-9.

18. Pucilowska JB, Davenport MB, Kabir I, Clemmons DR, Thissen J-P, Butler T, Underwood LE. The effect of dietary protein supplementation on insulin-like growth factors (IGFs) and IGF-binding proteins in children with shigellosis. J Clin Endocrinol Metab 1993;77:00-00.(in press)

Flow chart (sequence of task within time limit)

Organization of the study

1. Staff recruitment: 1 month [April 1-30]

2. Training of staff: 2 wks [May 1-14]

3. Pretesting: 2 wks [May 15-28]

4. Actual study:

   (a) Dietary intervention - 90 pts. Average 2 pt in a week. June 01, 1994 through May 31, 1995.

   (b) Stable isotope study will begin in June and will continue up to 3 months i.e. August '94.

   (c) Dr. D. Halliday (Co-investigator) from London, UK will visit the Centre for 2 weeks [June 1-14] to start-up isotope study in first few weeks.

5. **Follow-up study** will be completed by November 30, 1995.

6. Laboratory assay: Dr. Iqbal Kabir (PI) will be visiting Dr. Halliday's laboratory in UK to learn the assays for one month. November 15, 1995 to December 15, 1995.

7. Data entry and analysis and write up report: January 01 to March 31, 1996.

Itemized specific task for each listed investigators

1. Dr. Iqbal Kabir (PI): Overall responsibilities — Development and write up and protocol, conduction of the study, analysis of data and also to take clinical care in the study ward.

2. Dr. L.E. Underwood: Insuline-like growth factors (IGF) and IGF-binding proteins will be assayed in Dr. Underwood's laboratory at the University of North Carolina at Chapel Hill, USA (no financial implication for his salary).

3. Dr. Dave Halliday, London, UK: Stable isotope assay will be done with GC mass spectrometry at Dr. Halliday's institute. Dr. Halliday is working in this field for the last 15 years and has published more than 150 papers. He will visit ICDDR,B Dhaka to start the isotope study at the beginning of the study. He will receive only travel expenses and per diem in Dhaka.

REVISED BUDGET FOR THE PROJECT ENTITLED "THE EFFECTIVENESS OF VEGETABLE PROTEIN FOR REFEEDING MALNOURISHED CHILDREN RECOVERING FROM SHIGELLA".

[Figures are in US Dollar]

Personnel Cost

Name of position	No. of position	% of effort	Rate of monthly sal.	Total
Principal Investigator [Dr Iqbal Kabir]	1	50%	1450	17,300
Medical Officer [for pts recruitment, care etc.]	1	100%	545x24 m	13,100
Health Assistants [for anthropometry, data collection, follow-up home visit, etc.]	3	100%	210x3x24 m	15,120
Data entry technician [for data entry]	1	100%	210x12 m	2,520
Health Worker [to assist in feeding & diet preparation]	2	100%	40x3x24 m	2,880
Sub-total:				50,920

Local travel: [for follow-up home visits of study pts]

90 pts x 12 visits x $ 3/visit		3,240
Sub-total:		3,240

International travel + allowances

Dr Haliday

Ticket cost [London-Dhaka-London]:	1,650	
Per diem in Dhaka for 20 days @ $162/d x 20=	3,240	
Others (transportation etc. at Dhaka)	100	4,990

Dr Iqbal Kabir for short-term training for a month:

Ticket cost [Dhaka-London-Dhaka]	1,650	
Visa fee (multiple entry) at Dhaka	62	
Travel tax, embarkation fee etc. at Dhaka	50	
Per Diem in London for 30 days @ $206/d x 30=	6,180	8,042
Sub-total:		13,032

Supplies and materials:	
Stationery & office supplies	2,500
Laboratory supplies such as Vacutainer, EDTA vials, drum vials, naso deudenal tube, puc bags, vein cath. etc.	3,000
Hospital supplies, drugs etc.	2,000
Clearing & forwarding	500
Sub-total:	8,000

Laboratory investigations (local):

Name of investigation	Total test	Rate	Total
CBC, Hct	90	2.60	234
Stool microscopy	90	1.65	149
Stool culture	90	5.10	459
Serum total protein	90 x 2	0.77	139
Serum albumin	90 x 2	2.92	526
S. retinol binding protein	90 x 2	14.30	2574
Serum zinc	90 x 2	5.23	942
Stool nitrogen	90	11.5	1035
Stool calorie	90	9.7	873
Stool a1-antitrypsin (to evaluate mucosal healing)	90 x 2	20.5	3690
Food vitamin A	5 x 3	12.1	182
Food zinc estimation	5 x 3	5.23	79
Miscellaneous investigation for follow-up pts.			2000
Sub-total:			12,882

Stable isotope study:
Calculation based on 15 kg child, 6-hr infusion plus 1-hr prime

Isotopes	
[1-^{13}C]-leucine	900
[$^{2}H_3$]-leucine	4,200
[^{13}C]-bicarbonate	50
Analyses	
60 x 10 plasma leucine[a]	9,000
60 x 5 urinary leucine	4,500
10 x 18 enrichment calibration curbes	1,200
60 x 10 $^{13}CO_2$ expired air[b]	4,800
Sub-total:	24,650

[a]Reaction vials, septa x 2, tubes & derivatizing reagents, GC capillary column

[b]Vacutainer tubes, liquid nitrogen

Patient hospitalization cost: [90 pts x 26 d x US $21/d]	49,140
Plus dropouts, calculated as 15% dropout on or before study day 13	3,686
Sub-total:	52,826

Centre's interdepartmental services:	
Land transport (Dhaka)	300
Xerox & mimiography	500
X-Ray	500
Medical illustration	200
Telex/fax/telephone etc.	1,000
Transport subsidy (for staff members of the project)	1,000
Sub-total:	3,500

Other contractuals:

Rent, communication & utilities	500
Service charges	500
Printing & publication	1,000
Transportation cost for samples	1,000
Sub-total:	3,000

Equipments:

Harvard infusion pump: 2 pcs x 1750 (for isotope study)	3,500
Approx. airfreight	1,000
Microcomputer (one set) [Model MSQ 486DX-33C, Ram 8 MB, HDD 200 MB]	3,000
HP Laserjet 4 printer (one set)	2,500
Sub-total:	10,000

Budget summary

1.	Personnel	50,920
2.	Local travel	3,240
3.	International travel	13,032
4.	Supplies & materials	8,000
5.	Laboratory investigations	12,882
6.	Stable isotope study	24,650
7.	Patient hospitalization	52,826
8.	Interdepartmental service charges	3,500
9.	Other contractuals	3,000
10.	Equipments	10,000
Total:		182,050
Indirect cost (31%)		56,436
Grand total:		238,486

Justification of budget

- One Medical Officer will be recruited to scrutiny the study patients from out-patient department, take overall responsibility for patient care activities. He will also help the PI in data entry, data analysis etc.
- 3 Health Assistants will be recruited for selection of patients as per recruitment criteria, measurement of anthropometry, management of diets of study patients, data entry, follow-up home visits etc.

Travel & Consultation: An amount of US $13,032 has been allocated for this purpose. As isotope study is a new area of nutrition research and ICDDR,B does not have expertise, Dr. D. Halliday will come to the Centre for two weeks to help the PI to start up the isotope study. Dr. Iqbal Kabir (PI) will also visit Dr. Halliday's Laboratory to learn the assay method of "stable isotope". This is very important as we speculate by next few years many basic nutrition research will involve stable isotope studies. We shall be pioneer in this area if we can transfer the isotope technology to a developing country like Bangladesh.

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Table 1. Composition of nutrients of 3 study diets

1. Legume-based high-protein diet (per 1000 Kcal).

Food items	Amount(g)	Protein (g)	Fat (g)	Energy (Kcal)
Rice	112	6.72	-	392
Lentil (dal)	112	28.0	-	392
Oil (soybean)	24	--	24	216
		34.72	20	1000

Calcium 7.8 mg, iron 0.88 mg, carotene 27.0 mcg, folic acid 1.8 mcg, zinc 0.47 mg

Protein-energy ratio 14%

Fat-energy ratio 18%

Cost per 1000 Kcal; Tk=5.70 US $ 14.3 cents

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2. Legume-based standard protein diet (per 1000 Kcal).

Food items	Amount(g)	Protein (g)	Fat (g)	Energy (kcal)
Rice	165	9.9	-	578
Dal (lentil)	26	6.5	-	91
Oil (soya)	37	-	37	333
		16.4	40	1002

Calcium 3.6 mg, iron 0.82 mg carotene 8.1 mcg folic acid 1.7 mcg, zinc 0.28 mg

Protein-energy ratio 6.4%

Fat-energy ratio 33.3%

Cost per 1000 Kcal Tk=4.50 US $ 11.3 cents

3. Animal protein based High-protein (per 1000 Kcal).

Food items	Amount (g)	Protein(g)	Fat(g)	Energy (kcal)
Bread	60	5.25	1.12	120
Egg	45	6.01	5.54	90
Rice	100	1.98	----	124
Chicken curry	50	8.18	6.18	110
Banana	60	0.8	----	60
*Special milk formulation	500	18.58	22.9	496
Total		40.8	35.9	1000

Calcium 1290, zinc 3.83 mg, iron 8.9 mg, vitamin A 2000 iu

Protein energy ratio 16%

Fat energy ratio 32%

Cost per 1000 kcal: Tk.30.00 = US $0.75

*Special milk (high-protein diet) contained whole-milk powder (125 g), egg white (50 g), sugar (50 g), and soybean oil (20 g).

4. Essential Amino Acid (gram) contents of Study Diets

	Arginine	Histidine	Lysine	Tryptophan	Phenyl alanine	Tyrosine	Methionine	Cysteine	Terionine	Leucine	Isoleucine	Valine
Diet 1[a] Total protein 34.72 g	3.107	0.921	2.207	0.344	1.586	1.218	0.461	0.422	1.298	2.708	1.543	1.884
Diet 2[b] Total protein=16.4	1.576	0.467	0.806	0.173	0.616	0.683	0.400	0.231	0.688	1.376	0.772	1.051
Diet 3[c] Total protein=40.8	2.059	1.071	2.687	0.512	1.357	1.588	1.956	0.637	1.743	3.435	2.254	2.507

[a]Legume-based high-protein diet

[b]Legume-based standard protein diet

[c]Animal protein-based high-protein diet

Abstract Summary for Ethical Review Committee

Shigellosis is a major cause of childhood malnutrition in many developing countries including Bangladesh. Previous studies at ICDDR,B have shown accelerated catch-up growth of the children fed a high-protein (animal), diet during recovery from shigellosis. Since the sources of dietary protein we used are too costly in most developing countries, we propose a low-cost plant-protein based diet which will attain similar rate of catch-up growth of malnourished children as previous studies with animal protein diet.

The result of this study will help us to determine whether the anabolic effects documented during refeeding with large amount of animal protein are critical or whether equivalent rates of catch-up growth are achievable when low-cost plant-protein based diets are used. Furthermore, the result will have tremendous public health importance if shown a low-cost, plant-protein diet has similar efficacy as of animal based high-protein diet.

1. The appropriate subjects for this study are children 2-5 yrs. Because these children suffer from shigellosis and its consequences such as malnutrition.

2. Physical risk to the study population include temporary discomfort associated with blood collection and nasogastric tube. Feeding stable isotopes does not produce any risk and safe in children.

3. Physical risk associated with blood collection and nasogastric tube will be minimized as much as possible. Indeed these are routinely done for sick patients and those who are severely anorexic.

4. Confidentiality of the data collected during this study will be maintained by coding the questionnaires and biological specimens with study identification numbers, all information will be stored in locked files.

5. Since the study subjects are minor children a written consent will be obtained from parent or legal guardian. For illiterate persons the objective of the study and consent form will be read out and thumb print will be obtained in front of a witness.

6. On admission and during study (hospital) clinical history and physical examination will be done by PI or fellow physician. This is routinely done in patient care. During follow-up a history regarding the illnesses will be recorded. Interview would not be more than 20 minutes.

7. The individual subject will get benefit by receiving clinical care, dietary supplementation to promote catch-up growth and treatment for any illness during follow-up. The general benefit includes if this study shows accelerated catch-up growth with a low-cost plant-protein based diet. It will have public health benefit for underprivileged malnourished children who can not afford expensive animal protein.

8. The study will use clinical records, blood, urine and expired air.

CONSENT FORM

Short-title: Vegetable-protein in Shigella study

Your child is suffering from blood dysentery. The present knowledge from different studies suggest that due to loss of blood, serum proteins children with blood dysentery develop malnutrition and growth stunting. Previous studies have shown that feeding a diet with higher amount of animal-protein caused better growth. However, animal protein is expensive, and may not be afforded by most people in developing countries. Therefore, we want to investigate whether a vegetable protein (rice+lentil) based diet has similar efficacy in enhancing better growth.

If you agree to allow your child to participate in this study, your child will be admitted into the study ward. Your child will be treated with appropriate antimicrobial for 5 days and thereafter will be fed with either a animal-protein-based diet or vegetable-protein-based diet for 21 days. To determine the absorption of vegetable-protein your child will be fed a stable isotope which is safe to the children and does not alter normal metabolism. For the purpose of measurement of serum proteins and isotopes we will draw about 5 ml of blood on admission and at discharge to see the protein status of your child. Your child will be visited by the field worker and body weight, height will be recorded for 3 months.

If you agree to participate and comply with follow-up please sign on this form. If you do not agree to participate your child will still receive standard care provided by the hospital.

_____

Signature of the
Investigator

_____

Signature/Thumb impression
of the guardian

অনুমতি পত্র

"সিলেলা গবেষনায় সবজি জাতীয় আমিষের ব্যবহার"

আপনার শিশু রক্ত আমাশয়ে ভুগছে। বর্তমানে বিভিন্ন গবেষনায় জানা গেছে যে, যে সমস্ত শিশুরা রক্ত আমাশয়ে ভুগছে তাদের পায়খানার সাথে রক্ত বের হয়ে যাবার ফলে শরীরে আমিষের অভাব হয় এবং শিশু অপুষ্টিতে আক্রান্ত হয়। এবং সেই সাথে তার লম্বা হওয়াতে ও ব্যাঘাত ঘটে। পূর্ববর্তী গবেষনায় দেখা গেছে যে, অতিরিক্ত মাত্রায় প্রানীজ আমিষ খাওয়ানোর ফলে শিশু আবার নিয়মিত বাড়তে থাকে। সুতরাং আমরা এখন দেখতে চাই যে, সবজী জাতীয় আমিষের ব্যবহারে শিশু ঠিক ওই ভাবে বাড়ে কিনা।

আপনি যদি এই গবেষনায় অংশ নিতে রাজি থাকেন তবে আপনার শিশুকে স্টাডি ওয়ার্ডে ভর্তি করা হবে। তারপর আপনার শিশুকে আইরিক জীবানু-নাশক ওষুধ দ্বারা পাঁচ দিন পর্যন্ত চিকিৎসা করা হবে এবং একুশ দিন পর্যন্ত প্রানীজ আমিষ অথবা সবজী আমিষ খাওয়ানো হবে। সবজী জাতীয় আমিষ খাবার কতটুকু হজম হচ্ছে তা জানার জন্য আপনার শিশুকে একটা ওষুধ (সিবল আইসোটোপ) খাওয়ানো হবে – যা শিশুর জন্য নিরাপদ এবং স্বাভাবিক হজম ক্রিয়ায় কোন পরিবর্তন ঘটায় না। রক্তে আমিষের অবস্থা জানার জন্য আপনার শিশুর শরীর থেকে ভর্তির সময় এবং ছুটির সময় পাঁচ সি সি (এক চামচ) পরিমান রক্ত পরীক্ষার জন্য নেয়া হবে। আপনার শিশুকে বারবার যাতে সুচ ফুটাতে না হয় সেজন্য শিশুর হাতের শিরায় প্লাস্টিকের একটা সুই দিয়ে চার বারে আধা (½) মি: লি: রক্ত পরীক্ষার জন্য নেয়া হবে। এছাড়াও পরীক্ষার সময় বাচ্চা যাতে বমি করে ওষুধ না ফেলে দেয় সে জন্য একটা সরু নল পেটে ঢুকিয়ে খাওয়ানো হবে।

আপনি যদি এ গবেষনায় রাজি থাকেন তবে নিচে টিপ / সই দিন। আর আপনি যদি রাজি নাও থাকেন তথাপি হাসপাতালে আপনার শিশুর যাবতীয় চিকিৎসা এবং যত্ন ঠিকই থাকবে এবং আপনি যে কোন সময় আপনার শিশুকে গবেষনা থেকে প্রত্যাহার করে নিতে পারবেন।

গবেষকের স্বাক্ষর

অভিভাবকের স্বাক্ষর / টিপ সই