THE EFFECTIVENESS OF VEGETABLE PROTEIN DIET FOR REFEEDING MALNOURISHED CHILDREN RECOVERING FROM SHIGELLA

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Abstract

Shigellosis is a major cause of childhood mortality in developing countries. A substantial proportion of children who survive develop secondary protein-energy malnutrition (PEM) and become stunted. In a previous study at ICDDR,B using a high-protein (animal) diet with generous portions of selected micronutrients, we were able to show accelerated rates of catch-up in weight and length gain, ie., to begin to reverse stunting. However, the dietary ingredients we used are costly and therefore the intervention is impractical. Therefore, the next step is to test the hypothesis that stunting can also be reversed by carefully formulated diets based on affordable ingredients. To test this hypothesis, we will use rice-legume-based diets in which the amino acid patterns are complimentary, and will supplement the diet to increase intake of key micronutrients which affect linear growth. The effects of the experimental diet will be compared with those of a standard diet recommended by WHO/FAO and with those of the diet we used previously, which was based on animal products and provided 15% of energy as protein and more micronutrients than the standard refeeding diet. We will measure growth by standard means, but will add measurements of protein anabolism to learn whether this is an early predictor of length gain.

The study will be conducted in ninety Bangladeshi children aged two to five years who will be hospitalized in a metabolic refeeding ward for three weeks. During the study, anthropometric measurements will be made on day one and at the end of the study. The studies of protein turnover will be done using the stable isotope tracer ^{13}C-leucine and $^{2}H_3$-leucine, which can be administered safely to children because of the fact the isotopes are neither radioactive 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. Isotopic enrichment will be measured in samples of urine, plasma and breath by gas chromatography-mass spectrometry and isotope ratio mass spectrometry.

The results of this study will determine whether diets based on common Bengali foods reverse stunting. Further, it will improve our scientific understanding of how nutritional factors affect the body so that growth stunting is reversed. It will also show whether the protein anabolism is useful as an early predictor of length gain.

1. SCIENTIFIC BACKGROUND AND SCOPE OF THE PROJECT

Shigellosis is a major cause of childhood mortality in developing countries. Approximately 300 million children are infected with shigellosis, about 650,000 children die annually due to shigellosis [1]. A substantial fraction of the survivors suffers from secondary protein-energy malnutrition (PEM). Most of the deaths are due to severe life-threatening complications such as haemolytic-uraemic syndrome [2], sepsis [3] hypoglycaemia [4], hyponatraemia and intestinal obstruction [5]. The risk of death increases substantially in patients with severe PEM.

The effects of Shigella infection on growth in Bangladeshi children were studied by Black *et al.* [6] and Henry *et al.* [7] who found children with shigellosis had significant reductions in linear growth compared to those who had watery diarrhoea. The growth retardation during diarrhoeal disease is due to the interaction of loss of appetite and/or food withholding, malabsorption, increased catabolism of body protein, and to loss of serum proteins in the stool [8]. In severe shigellosis, as much as 500 ml of serum may be lost per day [9,10]. This leaking of serum protein may persist even during recovery [11], leading to or exacerbating malnutrition. In Bangladeshi children with severe shigellosis, serum protein concentrations are only half of the normal value [2]. In severe malnutrition, depressed serum albumin concentrations are instrumental in lowering plasma oncotic pressure and allowing the oedema of kwashiorkor to develop [12].

The conditions of optimal feeding during acute diarrhoea are still debated widely. Brown and MacLean [13] reviewed the fundamental issues in this debate. The theoretical advantages of delayed feeding include 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 continuation of breast feeding during diarrhoea. It has also recommended that the weaning diet be provided as it was prior to the onset of disease, with the exception that cow's milk or formula milk diets be diluted [13]. These recommendations apply principally to watery diarrhoeas that affect the small intestine. Less attention has been given to refeeding after shigellosis and other invasive diarrhoeas that affect the colon [14]. Kabir *et al.* [15,16] recently 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 rate in those studies in the control group (7.5% of energy as protein) was almost equal to the NCHS standard, but the rate of linear growth in the children fed the high-protein (15% of energy) significantly exceeded the rate in the control children. We have also shown that successful dietary treatment with high protein diet is associated with increased levels of insulin-like growth factor 1 (IGF-1) and IGF-binding proteins [15]. Either or both of these could play a role in stimulating growth. These findings are important because they imply that dietary interventions can be applied to stimulate the anabolic drive and speed recovery from PEM [17]. The findings also suggest the involvement of IGF-1 and IGF-binding proteins in producing the anabolic effects and open many questions about the specific roles of the various nutrients in stimulating linear gain.

Increases in linear growth have also been observed in undernourished Bundi children of New Guinea who were fed a high-protein diet [18] and in malnourished Peruvian children [19]. An association between accelerated linear growth and the high-protein diet in both

groups of children was suggested by the observation that control children fed a comparable diet but with less protein gained a higher percent of body fat [18] or did not show similar acceleration in length gain [19]. Jackson [20] has also discussed the tendency toward more deposition of fat tissue at the expense of lean tissue under conditions of refeeding with a diet that is either qualitatively or quantitatively too low in protein. In a study in Colombia, diarrhoea was negatively associated with body length among unsupplemented children whereas diarrhoea had no effect on length in children who were supplemented with a diet in which protein provided 14-15% of energy [21]. Similar findings have been reported in Guatemala and Brazil [22]. In a recent study in Nigerian malnourished children, a plant-protein based rehabilitation diet showed satisfactory recovery in growth in association with increased concentrations of IGF-1 values which were comparable with those fed a milk-based diet [23,24].

Since the source of protein used by Kabir *et al.* [16] was animal foods which are too costly in most developing countries where PEM is common, the next step is to determine whether more low cost sources of protein and micronutrients can be substituted while maintaining the anabolic effects of the high-quality protein and to learn what mechanisms underlie the increased growth rate. Therefore, we propose a plant-based legume-cereal (lentil + rice) diet for refeeding children with shigellosis during recovery, as rice and lentils are the major source of energy and protein and are widely available in many developing countries.

1.1. Rationale for using legume-based protein diet

The experimental diet we used previously was based on animal protein which is too costly to be applicable widely in most developing countries where shigellosis and PEM are public health concerns. As in many developing countries, rice and dal (red gram/lentils) are combined in Bangladesh to make a dish known as khichuri. Rice is relatively deficient in lysine and lentils are relatively deficient in methionine. However, the combination produces a food with a complimentary amino acid pattern that is expected to be able to meet the known requirements for amino acids in growing children.

As the plant-based diet we propose may contain less zinc, which might be a limiting micronutrient for growth, lean tissue repletion [25] protein synthesis or muscle synthesis [26], additional zinc will be supplemented with the to match the intake in the animal-based high-protein group. Additional carotenoids, calcium, folic acid, and iron will also be provided as each of these is expected to affect length gain.

2. METHODS

2.1. Objectives.

To test the hypothesis that:

(1) Diets which provide approximately 15% of energy as protein and which are based on low-cost commonly available foods:

(a) Stimulate the anabolic drive and speed catch-up growth by accelerating weight gain and height accretion, increasing lean body

mass, and improving rates of net protein anabolism as evidenced by increased ratios of rates of protein synthesis to rates of protein breakdown.

(b) Increase the serum concentrations of total protein, pre-albumin, retinol-binding protein, IGF-1, and IGF-binding protein.

(2) Catch-up growth, measured by standard anthropometrics, can be predicted by favourable changes in the ratio of rates of protein synthesis to rates of protein breakdown.

2.2. Study Design

This study will be conducted in ninety preschool Bangladeshi children who will be hospitalized in a metabolic-refeeding ward at the ICDDR,B during the three week course of study. Subjects will be assigned randomly to one of three dietary groups (see Treatment protocol and Table) and will receive the experimental diet assigned to them for the duration of the study. Clinical, laboratory, anthropometric, protein kinetic, and body composition data will be obtained according to the methods and protocol described below. The laboratory and body composition data will be collected before the study diet is begun and again after the twenty-one day treatment and data analyzed by paired comparisons so each subject will serve as his/her own control. Anthropometric data will be collected more often and paired comparison will also be made of these data. Protein kinetic data will be collected as early as feasible, following an appropriate period of dietary equilibration, and again after the twenty-one day dietary therapy. Paired comparisons will also be made of these data.

2.3. Subjects

Children aged twenty-four to sixty months whose parent or guardian consent to the child remaining in the hospital for three weeks will be admitted if they meet the criteria listed below. At least one patient per week can be admitted during the two year period of the study.

2.3.1. Admission criteria

(a) History of blood-mucoid stool for less than five days
(b) Stool culture positive for *Shigella sp.*
(c) Length-for-age <-3.0 Z scores by NCHS
(d) Weight-for-length < -1.5 Z score by NCHS
(e) No frank kwashiorkor
(f) No complicating illnesses such as pneumonia, tuberculosis, septicaemia
(g) No haemolytic-uraemic syndrome
(h) Parental consent

2.4. Sample size calculation

From the NCHS standards and from our previous study [16] in children aged between twenty-four and sixty 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 nets of 2 mm per month in height, the required sample size is twenty-eight in each group. Calculating for dropouts the total sample size is ninety patients, i.e., thirty in each group. However, to determine the protein kinetics using deuterated and ^{13}C-leucine, only ten patients are needed in each group.

Identification and enrolment of children: Children suspected of having shigella will be identified by the health workers in one of the clinics associated with the ICDDR,B and referred to the study team for further evaluation.

2.5. Initial examination

2.5.1. Clinical

On admission a clinical history and physical exam will be completed initially to assure that each patient fulfils the entry criteria for the study. This information obtained on admission will also be used *post-hoc* to compare the two treatment groups, to assess their initial similarity. The history and physical exam 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 in appetite.

(c) Diet during the previous twenty-four hours.

(d) Treatment provided prior to admission, including oral rehydration solutions and medications.

(e) Physical examination for degree of dehydration, nutritional status, and any pathophysiological physical findings.

(f) Weight, height, midarm circumference, triceps, biceps, suprailiac, and subscapular skinfolds, and head circumference will be recorded.

2.5.2. Laboratory

On admission, analyses of stool and blood culture will be performed to assure that each patient fulfils the entry criteria:

(a) Stool microscopical examination for faecal leukocytes, red blood cells count and sensitivity.

(b) Haematocrit, and complete blood count.

(c) Venous blood will be analyzed for serum proteins, serum zinc, IGF-1 and IGF-BF.

The measurements will be repeated as needed in any patient who manifests clinical signs of diarrhoea or 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 and IGF-BP will be determined at Dr. L.E. Underwood's laboratory at the University of North Carolina, using standard technique as previously described [15].

2.6. Diets

The diets will be either animal-based high-protein, diet (15% of energy as protein, derived from animal protein), or a vegetable-based high-protein diet with supplemental intake of calcium, zinc, iron, folic acid, and carotenoids, (15% of energy as protein, grain/legume-based), or a WHO/FAO reference diet for undernourished children (7% of energy as protein, derived from vegetables). The dietary treatments will be provided during the entire twenty-one day study period. The diet will be prepared in a semi-liquid consistency at a dilution of 75 kcal/100 ml and will be provided ad libitum up to a maximum of 150 kcal (200 ml/kg) body weight per day in six feedings. Intake will be determined by weighing the feeding vessels before and after each feeding and recording the weight of spills and/or food regurgitated. Diet composition is shown in Tables I, II, and III.

2.7. Randomization

After inclusion in the study the children will be randomly assigned to either the animal-based diet, to the vegetable-based diet (15% of energy as protein, grain/legume-based), or to the reference standard diet (7% of energy as protein, derived from vegetables). Random assignment to study group will be made using sequential numbers from a random number table. The sequential numbers will be kept in sealed envelopes and will be opened just before the study begins.

2.8. Antimicrobial and dietary treatment

All children will be treated prior to the study with appropriate antimicrobial (pivmecillinam) for five days. The effectiveness of the treatment will be assessed clinically as (1) the absence of fever; (2) absence of straining during defecation; (3) absence of blood and mucus in stool; (4) reduction of stool frequency to less than four stools per day.

2.9. Body Composition

2.9.1. Anthropometry

Body weight will be obtained on admission and daily at 09:00h on a SECA scale (Seca Scale Co., Germany, Model 72 W) with a precision of 5 g, the height will be measured on a length board on admission and every third day until the study is over, and thereafter every fifteenth day during follow-up. At least three measurements will be obtained by trained health assistants. Mid-arm circumference, and skinfolds will be measured on admission and after seven, fourteen, and twenty-one days of nutritional therapy using standard techniques.

2.9.2. Bioelectrical impedance

To monitor the impact of supplementing the different study diets on body composition, bioelectrical impedance assay will be done in children before the study begun and at the end of twenty-one day study period using a BIA analyzer (BIA, RJL model 101A). 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 of height and body weight changes with fat free mass (Kabir *et al.* AJCN submitted).

2.10. Protein kinetics

Protein kinetic studies will be performed on a subset of each dietary group before and following the twenty-one day dietary therapy. [1-^{13}C] leucine and [$^{2}H_3$] leucine (1 mg.kg^{-1}.h^{-1})] will be infused by intravenous and nasogastric routes, respectively, for four and six hours, respectively. The intravenous infusions will be prefaced by priming doses of [1^{13}C] leucine (1 mg.kg^{-1}) and ^{13}C-bicarbonate (0.18 mg.kg^{-1}) [27]. The nasogastric infusions will be by tube into the small intestine (aspirate pH checked). Protein kinetic studies will be conducted following an eight hour overnight fast, or in the face of small repetitive oral feeds (every thirty minutes) to mimic energy expenditure at the resting metabolic rate. The resting energy expenditure will be measured in the "postabsorptive" state which, for practical reasons, may not be longer than six hours after a feeding. The REE will be measured for $\geq$30 minutes, during which the child is quiet and in thermal neutral and quiet environment on a day immediately prior to the infusion study. Gas exchange equipment already available at the ICDDR,B can be used for these measurements. The principal investigator and staff at the ICDDR,B are experienced in the proper use of the equipment for making determinations of respiratory gas exchange and REE measurements. Calculation of protein kinetics i.e., rates of protein synthesis, breakdown and oxidation, will be based on measurements of the enrichment of labelled leucine and its ketoacid (ketoisocaproate) [28] in plasma and in 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 of label (baseline) and at fifteen minute intervals over the last two hours of the infusions. The intravenous infusions of [1-^{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 [29]. Importance is attached to studies in the 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 in the fasted state.

2.11. Treatment failure

Children with recurrence of dehydration $\geq$ 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 introduced *ad libitum* at full concentration until the specific upper limits on volume or energy intake are achieved. Elevated blood urea nitrogen will also be criterion for interrupting the study, since this may suggest excess nitrogen intake.

This is unlikely, however, since the diets in the previous protocols with comparable levels of dietary protein were well tolerated [15,16].

2.12. Data analyses

Increments in weight ($g.kg^{-1}.d^{-1}$) and height (mm per day actual versus mm/d predicted by NCHS for height-age) will be calculated and compared among the groups. Increments in height and weight will be compared by Student's paired t-tests. Laboratory, anthropometric, protein kinetic, and body composition data will be compared early and late in recovery by treatment group using analysis of variance (ANOVA). The NCHS-CDC statistics package will be used to compare the Z-score values for anthropometry. The clinical indices of diarrhoea and other infections will be compared by Fishers Exact test, Chi-Square test.

3. SIGNIFICANCE AND INTERNATIONAL NUTRITION IMPLICATION

Protein-energy malnutrition (PEM) remains a major public health problem in most developing countries. The magnitude of this problem is more obvious in underprivileged community in least developed countries like Bangladesh. Bangladesh Bureau of Statistics in their recent survey (1991) found that about 35% of under-five children were severely stunted (below -3 SD). About 40% of children were both severely undernourished and stunted. The causes of this PEM are multifactorial and perhaps related to several factors including improper weaning practices, recurrent infections, poverty and also inadequate diet during and after infection. Therefore, there is an urgent need to develop and formulate a designer food which can be supplemented to prevent growth faltering and stimulate linear growth recovery of children suffering from recurrent infections with secondary PEM. The results of this study are needed 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 diets either as they are normally prepared or with the addition of micronutrients known to affect linear growth are used. These studies 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 which is measured classically 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. If an appreciable anabolic effect of the vegetable-based diet can be demonstrated, we plan additional studies of the nutritional regulation of the anabolic drive during refeeding of undernourished children.

4. COLLABORATIVE ARRANGEMENTS

This study will be carried out at the ICDDR,B in collaboration with Dave Halliday, PhD, Head of the Nutrition Research Group, Clinical Research Centre, Watford Road, Harrow, Middlesex He 300, UK, Dr. L.E. Underwood, University of North Carolina at

Chapel Hills, NC, USA (for measuring IGF and IGF-BP study), and Carla R. Fjeld, PhD, Senior Scientist at the International Atomic Energy Agency.

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TABLE I. COMPOSITION OF NUTRIENTS OF THREE STUDY DIETS

DIET I. LEGUME-BASED HIGH-PROTEIN DIET PER 1000 KCAL

Food items	Amount (g)	Protein (g)	Fat (g)	Energy (kcal)
Rice	115	28.75	--	403
Lentil (dal)	115	28.75	--	403
Oil (soybean)	23	--	23	207
		---	---	---
		3.1	23	1013

Calcium, 7.8 mg; Iron 0.88 mg; Carotene, 27.0 μg; Folic acid, 1.8 μg; Zinc, 0.47 mg.

Protein-energy ratio 15%

Cost per 1000 kcal Tk. 5.75 = US $ 0.14

DIET II. LEGUME-BASED STANDARD PROTEIN DIET PER 1000 KCAL

Food items	Amount (g)	Protein (g)	Fat (g)	Energy (kcal)
Rice	170	11.9	--	595
Dal (lentil)	30	7.5	105	360
Oil (soya)	40	--	40	360
		---	---	---
		19.4	40	1060

Calcium, 3.6 mg; Iron 0.82 mg; Carotene, 8.1 μg; Folic acid, 1.7 μg; Zinc 0.28 mg.

Protein-energy ratio 7.3%

Cost per 1000 kcal Tk. 4.50 = US $ 0.11

DIET III. ANIMAL PROTEIN BASED HIGH-PROTEIN (PER 100 G) DIET

Food items	Protein (g)	Fat (g)	Zinc (mg)	Energy (kal)
Bread	8.75	1.86	0.1	2.6
Rice	1.98	0.15	0.33	124
Chicken Curry	16.36	13.6	0.72	222
Egg	13.23	12.31	1.26	200
*Special Milk Formulation	3.72	4.58	0.55	99
Banana	1.42	0.19	0.14	100

Protein-energy ratio 16%

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).