Original Article

Lentil-based high protein diet is comparable to animalbased diet in respect to nitrogen absorption and nitrogen balance in malnourished children recovering from shigellosis

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Previous studies showed better absorption of protein and catch-up growth with animal-based high protein (15% energy from protein) diets (AP) than plant-based diets. This study compared the intake and absorption of nutrients from a lentil-based high protein (15% energy from protein) diet (LenP), AP, and a low protein (7.5% energy from protein) diet (LP). A total of 31 moderately malnourished 24 to 59 month old children convalescing from shigellosis were randomised to these three diets: LenP (n=11), AP (n=9) and LP (n=11). After two weeks adaptation with the respective diets, a 72-hour metabolic balance study was performed. The children's baseline characteristics were comparable among the groups (one exception: children of LP group were less stunted). The costs of 1,000 kcal from LenP, AP and LP diets were 0.15, 0.75 and 0.11 US dollar, respectively. Average daily energy intake (115-119 kcal/kg/d), coefficients of carbohydrate (89-91%), fat (80-90%), and energy (87-89%) absorption were similar in all groups. Mean±SD coefficient of nitrogen absorption (%) and nitrogen balance (g/kg/day) were 81±6 and 0.35±0.21 in LenP, 82±5 and 0.36±0.08 in AP, and 73±4 and 0.13±0.06 in LP groups, respectively (for both the nitrogen absorption and balance comparisons: LenP vs. AP, p>0.05; LenP vs. LP, p<0.05; AP vs. LP, p<0.05). The results showed higher absorption of nitrogen and its balance from high protein diets whether derived from lentil or animal source, which may enhance tissue protein deposition. A lentil-based high protein diet, which is less expensive, may be useful for nutritional rehabilitation of moderately malnourished children.

Key Words: lentil-based high protein diet, animal-based high protein diet, nitrogen absorption, nitrogen balance, malnutrition

INTRODUCTION

In developing/low-income countries diarrhoeal diseases and under-nutrition are major health problems in children under 5 years of age. Shigellosis is a common cause of dysenteric illness in this age group and is associated with growth faltering, particularly stunting in young children.^{1,2} Anorexia, increased catabolism, malabsorption and nutrient loss, in addition, the practice of food withholding during shigellosis worsen the nutritional status.³⁻⁸ Previous studies by Kabir et al. showed better absorption of protein,⁹ improvement of retinal binding protein, other serum proteins, and vitamin A,¹⁰ and enhanced catch-up growth during convalescence from Shigellosis¹¹ as well as after six months of follow-up¹² with an animal-based high protein diet. Since animal protein is expensive in most developing/low-income countries and cannot be afforded by underprivileged communities whose children are most likely to suffer from malnutrition, the recommendation of animal-based high protein diet may have limited application. Nigerian malnourished children who were fed a vegetable protein rehabilitation diet showed a satisfactory recovery in growth.¹³ A trial with a low cost and locally available plant-based high protein diet is worthwhile. We hypothesized that high protein diets, whether of plant (lentil) or animal (cow's milk and chicken) origin have comparable effect in protein absorption. For this purpose a legume-cereal based diet was used for re-feeding children convalescing from shigellosis. Rice and dal (red gram/lentil) are the major sources of energy and protein widely available throughout Bangladesh and many other low-income countries. Although rice is deficient in lysine and lentils are deficient in methionine, combining these two makes a balanced diet with all essential amino acids that is likely to meet daily requirements. To determine the intake of macronutrients and to

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quantify absorption of nutrients from high protein ($\sim 15\%$ energy from protein: lentil-based and animal-based) diets, and low protein diet with $\sim 7.5\%$ energy from protein we have conducted this metabolic balance study.

MATERIALS AND METHODS

Study design

It was a randomised clinical trial with three different diets: 1) lentil-based high protein diet (LenP), 2) animal-based (cow's milk, egg and chicken) high protein diet (AP), and 3) low protein diet (7.5% energy from protein; lentil as the main source of protein; usual diet for poor communities in Bangladesh) (LP). (Table 1)

Subjects and settings

The study was done in the metabolic study ward of Dhaka Hospital of ICDDR,B during 1996 to 1999. Both boys and girls aged 24 to 59 months with moderate undernutrition and culture-proven shigellosis were enrolled in the study. Children with severe under-nutrition (weight for age < 60% of the median of National Centre for Health Statistics) and/or complications were not enrolled. All the children were treated with syrup pivmecillinum 15 mg/kg every six hourly for five days and all Shigellae isolates from stool specimen were sensitive to pivmecillinum. After clinical recovery from shigellosis, on day-six of the hospital admission, children were randomised to any of the above-mentioned three types of diet. Random assignment to a study diet was made using sequential numbers from a random number table. The sequential number with which the diet was assigned was kept in a sealed envelope and was opened just before the enrolment of each child. Informed consent to participate in the study was obtained from the parents of the children. A predesigned questionnaire was filled out, and trained health assistants did the anthropometry. The study was approved by the Research Review Committee and the Ethical Review Committee of ICDDR,B.

Diet

Table 1 describes details of the diet used in this study.

Different food items were offered ad libitum up to 150 kcal/kg/day divided in six meals and the meals were tailored so that the children got energy from protein according to diet group (LenP: 15%, AP: 15%, and LP: 7.5%). Because the lentil (legume)-protein based diet might be low in zinc, children who were under LenP and LP diet groups received syrup zinc acetate orally 20 mg/day. Every meal was pre-weighed to the nearest 1 g on a tabletop balance (Ohause Scale Co, Florsham, NY, USA) before each feeding, and the leftover food and vomitus (if any) were measured and subtracted to calculate the intake. To determine the actual intake of nitrogen, fat, carbohydrate, and energy, duplicate diets were prepared on three occasions for each dietary group and macronutrient contents were assayed in the laboratory. The mean values were used to compute the actual intake of nitrogen, fat, carbohydrate, and energy for all subjects.

Metabolic balance study

After two weeks of adaptation with the study diet, a 72hour metabolic balance study was done. All meals and supplements were provided under direct supervision of trained health assistants. Children were weighed to the nearest 10 g on a digital weighing balance (Ohause Scale Co) each morning between 8:30 to 9:00 AM after their first void of urine and before breakfast. During the balance period urine was collected in an adhesive-backed paediatric urine collection bag, and stool was collected in a pre-weighed can with graduation marks and measured once in every 8-hour interval; a sample was kept. A charcoal marker (first marker) was fed to the child and from the time of appearance of the first marker in stool, all stools were collected till the appearance of the second marker, which was fed 72 h after the first one. The time interval between the feeding of the charcoal marker and its appearance in the stool was the intestinal transit time. All faecal and urine samples were stored at -20°C until assay. The total sample collected was blended with a known amount of water before analysis for nitrogen, fat, and total energy. Laboratory procedures were done at the biochemistry and nutrition lab of ICDDR,B and assayed

Table 1. Nutrient content (per 100 g) and cost of three different diets

Fact it	Lentil Protein (LenP)		Animal Protein (AP)		Low Protein (LP)	
Food item	Energy	Protein	Energy	Protein	Energy	Protein
	(Kcal)	(g)	(Kcal)	(g)	(Kcal)	(g)
Cooked rice	124	1.98	124	1.98	124	1.98
Banana	100	1.4	100	1.4	100	1.4
Vegetable	48	1.43	48	1.43	48	1.43
Chicken curry	-	-	222	16.36	-	-
Whole egg	-	-	200	13.4	-	-
Special milk [†]	-	-	99.1	3.71	-	-
Liquid dal (lentil)	-	-	-	-	121	1.63
Thick dal (lentil)	134	5.32	-	-	-	-
Roasted dal (lentil)	380	27.5	-	-	-	-
Snacks (moa: puffed rice with molasses & peanut)	377	6.9	-	-	377	6.9
Percent of energy from protein	15		15		7.5	
Cost per 1,000 al (US \$) ^{††}	0.15		0.75		0.11	

⁷Special milk contained whole-milk powder, rice powder and soy oil to make 15% of the

calories from protein

^{††}1 US = 50 Taka (average) during the study period (1996-99)

	Lentil protein	Animal protein	Low protein
	(LenP)	(AP)	(LP)
	(N = 11)	(N = 9)	(N = 11)
Age, (month)	43.3 ± 9.5	42.8 ± 10.9	35.2 ± 12.2
Boy / Girl (n)	6 / 5	5 / 4	7 / 4
Weight (kg)	10.5 ± 2.0	10.4 ± 1.6	10.4 ± 1.4
Height (cm)	89.6 ± 7	88.4 ± 5	88.0 ± 7
Weight for age Z score (NCHS ^{\dagger})	-2.96 ± 0.9	-2.97 ± 0.7	-2.54 ± 0.7
Height for age Z score (NCHS)	-2.35 ± 1.4	-2.54 ± 0.9	$-1.43 \pm 1.1^{\dagger\dagger}$
Weight for height Z score (NCHS)	-2.19 ± 0.6	-2.10 ± 0.7	-2.04 ± 0.5
Mid upper arm circumference (cm)	13.7 ± 0.9	13.4 ± 1.4	13.6 ± 0.7
Triceps skin fold thickness (mm)	6.2 ± 0.8	5.8 ± 1.1	5.8 ± 0.8
Preadmission dysenteric illness (day)	3.6 ± 1.5	3.8 ± 1.6	3.7 ± 1.5
Preadmission stool frequency /24h	23 ± 10	21 ± 12	26 ± 16
Straining (n)	11	9	11
Anorexia (n)	11	9	9
Fever (rectal temperature $> 38^{\circ}$ C (n)	4	4	4
Stool RBC >50 /HPF (n)	10	9	8
Stool WBC >50 /HPF (n)	11	9	10
Stool pathogens (n)			
S. dysenteriae type-1	6	6	2
S. flexneri	4	3	6
Other Shigella spp.	1	0	3

Table 2. Clinical characteristics and laboratory features of children on admission/baseline

[†]National Center for Health Statistics

Values are mean \pm SD, unless mentioned otherwise and comparable among the groups (^{††}except height for age Z score: LP vs. LenP: *p* <0.05, and LP vs. AP: *p* <0.05)

for nitrogen concentration by the micro Kjeldahl method¹⁴ and for faecal fat by a modified Van De Kamer method,¹⁵ the energy contents of the diet and stool samples were determined by adiabatic bomb calorimetry, comparing the samples to a benzoic acid standard. The carbohydrate contents of diet and stool were calculated by subtracting the energy from nitrogen and fat from the total energy. The formula used for estimating carbohydrate was:

[Total energy (kcal)-{(N(g)*6.25*5.65} + {F(g)*9.40}] /4.15, Where, 5.65, 9.40, and 4.15 represent the kcal contained within 1 g of protein, fat, and carbohydrate, respectively.¹⁶ The coefficient of absorption of nutrients was calculated by using the following formula:

Coefficient of absorption (%) =

Apparent nitrogen absorption was calculated by subtracting the faecal nitrogen from the actual intake and expressing the difference as a percentage of the actual intake.¹⁷ Apparent nitrogen balance (retention) was calculated similarly by subtracting the urinary nitrogen from the apparent nitrogen absorption. Estimation of protein balance was done by multiplying the nitrogen value by 6.25.

Data analysis

Data were entered in a personal computer and analysis was carried out using the SPSS Windows (version 12.0; SPSS Inc, Chicago) software. Parametric data were compared by ANOVA followed by Tukey multiple pair wise comparisons. Nonparametric data were compared by the Kruskal-Wallis test followed by the Mann-Whitney U test. Categorical variables were compared by Chi-square test (the Fishers exact test was applied if the expected number in any cell was 5 or less). A *p*-value less than 0.05 was considered statistically significant.

RESULTS

A total 31 children were studied, of them 18 were boys and 13 were girls. Eleven, 11 and 9 children received LenP, AP and LP diet, respectively. To get an average 1,000 kcal from LenP, AP and LP diet the costs were 0.15, 0.75 and 0.11 US \$ respectively [1 US = 50 Taka (Bangladeshi currency) during the study period]. Table 2 shows the baseline clinical characteristics, nutritional status and laboratory features of the children, which were similar in all groups (except one: LP group was less stunted). Although, children in the LP group were a bit younger, age was not significantly different than the other two groups.

Macronutrient and energy intake in the study groups are shown in Table 3. The mean carbohydrate intake was greater in the LenP and LP groups compared to the AP group. The mean fat intake was highest in the AP and medium in the LP group. However, the mean protein (nitrogen) intake was significantly higher in the LenP and AP groups compared to the LP group. Although the average daily energy intake was similar (115 to 119 kcal/kg/d) in all the groups, the percentage of energy from protein was highest in the AP group and medium in the LenP group. Table 3 also shows the loss of macronutrient and energy through stool, and their coefficient of absorption. The coefficients of carbohydrate and energy absorption were similar in all the groups (89-91% and 87-89% respectively). The coefficient of fat absorption was apparently lower in LenP and AP groups, but did not significantly differ from the LP group. However, the coefficient of nitrogen absorption was significantly greater in the LenP and AP groups compared to the LP group. Although

	Lentil protein	Animal protein	Low protein
	(LenP)	(AP)	(LP)
	(N = 11)	(N = 9)	(N = 11)
Intake (per kg body weight /day)			
Carbohydrate (g)	20.3 ± 4.4^{a}	15.1 ± 1.9^{b}	$20.8\pm3.0^{\rm a}$
Fat (g)	$1.87\pm0.88^{\rm a}$	4.13 ± 0.74^{b}	$2.79 \pm 0.85^{\circ}$
Nitrogen (g)	$0.72\pm0.20^{\mathrm{a}}$	$0.83\pm0.16^{\rm a}$	$0.36\pm0.05^{\rm b}$
Energy (kcal)	$115.4 \pm 24.0^{\rm a}$	$118.5 \pm 17.0^{\mathrm{a}}$	117.5 ± 11.7^{a}
Percent of energy from protein	$12.6 \pm 1.4^{\rm a}$	16.4 ± 1.6^{b}	7.4 ± 0.5^{c}
Loss through stool (per kg body weight /day)			
Carbohydrate (g)	2.26 ± 1.05^{a}	1.36 ± 0.45^{b}	$1.79 \pm 0.59 a^{b}$
Fat (g)	$0.36\pm0.17^{\rm a}$	$0.68 \pm 0.53^{ m b}$	$0.31 \pm 0.23^{\mathrm{a}}$
Nitrogen (g)	$0.13\pm0.03^{\mathrm{a}}$	$0.14 \pm 0.03^{ m a}$	$0.10 \pm 0.02^{\rm b}$
Energy (kcal)	$14.2\pm7.0^{\mathrm{a}}$	$15.2 \pm 4.4^{\rm a}$	12.4 ± 3.5^{a}
Coefficient of absorption (%)			
Carbohydrate	$89 \pm 4^{\mathrm{a}}$	$91 \pm 3^{\mathrm{a}}$	91 ± 3^{a}
Fat	$80\pm7^{ m a}$	$83 \pm 13^{\mathrm{a}}$	$89\pm 6^{\mathrm{a}}$
Nitrogen	$81\pm^{6a}$	$82 \pm 5^{\mathrm{a}}$	$73 \pm 4^{\mathrm{b}}$
Energy	$88 \pm 5^{\mathrm{a}}$	$87 \pm 4^{\mathrm{a}}$	$89\pm3^{\mathrm{a}}$
Loss through urine (g/kg body weight /day)			
Nitrogen	0.26 ± 0.05^{a}	0.33 ± 0.14^{a}	0.13 ± 0.06^{b}
Nitrogen balance (g/kg body weight /day)			
[Intake-(stool loss + urine loss)]	0.35 ± 0.21^{a}	$0.36\pm0.08^{\rm a}$	0.13 ± 0.06^{b}

Table 3. Intake, loss through stool and coefficient of absorption of macronutrients and energy, loss of nitrogen through urine and nitrogen balance in three diet-groups

Values are mean \pm SD

Parametric data were compared by ANOVA followed by Tukey multiple pairwise comparisons

Nonparametric data were compared by Kruskal-Wallis test followed by Mann-Whitney U test

Values without a common superscript are significantly different with < 5% level of significance

loss of nitrogen through urine was highest in the AP and medium in the LenP group, nitrogen balance was comparable between the LenP and AP groups, but was significantly higher (in both the groups) than the LP group.

The results showed higher absorption of protein (nitrogen), and apparent nitrogen balance from high protein diets whether derived from plant (lentil) or animal source. This further suggested that the additional dietary protein led to a better nitrogen balance in children fed the highprotein diets, which may help in more deposition of tissue protein.

DISCUSSION

A previous study by us showed better absorption of protein with an animal-based (cow's milk, egg and chicken) high protein diet in children recovering from shigellosis.⁵ The recommendation of animal-based high protein diets has limited application since they are expensive and cannot be afforded by members of underprivileged communities whose children are most likely to suffer from malnutrition. In the present study the intake and absorption of carbohydrate, fat, nitrogen, and total energy were compared in three groups of diet fed to children recovering from shigellosis. The demonstrated lowest carbohydrate intake as well as highest fat intake in the AP group was due to the different dietary composition offered in this group. Because all groups were offered iso-caloric diets, and the AP group received more protein (protein energy ratio was ~15%) and the source of protein was animal (cow's milk, egg and chicken), the AP group received comparatively less carbohydrate as well as more fat in addition to protein from the diet. The coefficients of absorption of carbohydrate, fat, and total energy in all the

groups of our study were similar to those found in another study done in children recovering from acute shigellosis.¹⁸ Comparatively lower absorption of nitrogen/protein in that study might be due to different dietary composition.

However, the coefficient of apparent nitrogen absorption as well as apparent nitrogen balance were significantly higher in children fed both LenP and AP diets than in children fed the LP diet. This might be for any of the following reasons. Most of the children were moderately malnourished. Thus their total body protein was deficient. The groups received more protein irrespective of animal or lentil sources (i.e., AP and LenP groups) so the intestine might avidly absorb more nitrogen from the available protein to replenish lost/deficient protein.

The metabolic balance in our study was done during convalescence, when food intake was more likely to increase due to the return of appetite,¹⁹ even then the average total energy intake per day was not up to the expected level for children in any group. Our target was to provide ~150 kcal/kg/day, but the children had intakes of 115 to 119 kcal/kg/d. Like us, others have also reported limited food intake due to severe anorexia during acute diarrhoea. In their study, Hoyle et al.²⁰ found a reduction in food intake of about 30%. Molla et al.¹⁸ found a similar reduction of food/energy intake during acute shigellosis. The reason for the lower intake of food even during the convalescence period might be persistence of inflammation of the colon and anorexia. There could be some minor persistence of circulatory non-specific mediators such as interleukins, tumour necrosis factors, and cachectins, which are known to suppress the appetite centre.²¹

This study has important implication, because dysenteric illness due to shigellosis is very common in developing/ low-income countries, and is associated with malabsorption, nutrient loss, increased catabolism and growth faltering in young children.^{1,2} Children usually suffer for a prolonged time if they have an attack of shigellosis, and as many as 16% episodes of shigellosis lasted more than 3 weeks in Bangladesh.¹ A similar proportion lasted more than 2 weeks in Peru.²² Children may lose a substantial amount of protein during an acute episode of severe shigellosis.^{7,23} In addition, loss of immunoglobulins, lymphocytes, and granulocytes may depress immunity and can make the child more vulnerable to recurrent infection.^{24, 25} If the loss of such proteins in the gut results in diminished serum concentrations, it may contribute to a number of observed complications of shigellosis including increased susceptibility to infections^{26,27} and undernutrition.^{1,2} Many children in developing countries receive no effective therapy or are treated late in the course of their illness. It is likely that in such patients continued gut protein loss will have a more adverse effect on serum protein concentrations, especially in undernourished children, in whom serum proteins and amino acid concentrations are already low.²⁸ Therefore, it is desirable to replenish these losses with a diet adequate in protein and energy to promote catch-up growth. A low cost and locally available plantbased (e.g., lentil) high protein diet is a practical choice in developing/low-income countries, and its absorption is examined in this study.

The results of this study showed better absorption of nitrogen from high protein diets whether from lentil or animal (cow's milk, egg and chicken) sources. A high protein diet based on lentils, which is less expensive than diets based on animal protein, may be useful for nutritional rehabilitation in developing/low-income countries, where most of the people are poor and cannot afford animal protein diets.

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AUTHOR DISCLOSURES

None of the investigators has any financial interests that might be affected by the results of this study.

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以扁豆為主的高蛋白質飲食與動物性蛋白質飲食有相近 效果:觀察從桿菌性痢疾復原的營養不良兒童之氮吸收 及氮平衡

之前的研究顯示動物性蛋白質來源的飲食(AP,15%熱量來自蛋白質)比起植物性來源的飲食,有較好的蛋白質吸收率及促進生長。這個研究比較一個以扁豆(LenP)為主的高蛋白飲食(15%熱量來自蛋白質),AP及一個低蛋白質飲食(LP,7.5%熱量來自蛋白質)的營養素攝取及吸收能力。年齡24-59個月大的31名輕度營養不良,由桿菌性痢疾復原的兒童,隨機分成三種飲食組:LenP(n=11)、AP(n=9)及 LP(n=11)。經過兩週對於個別飲食的適應期後,進行一個72小時代謝平衡研究。各組的兒童基本特性都相似(惟一例外:LP 組兒童的身高不足差距較小)。來自 LenP、AP及 LP 飲食的 1,000 卡價格分別為 0.15、0.75及 0.11 美元。每日熱量攝取平均值(115-119 kcal/kg/d)與碳水化合物(89-91%)、脂肪(80-90%)及熱量(87-89%)的吸收係數各組都相似。氮吸收(%)及氮平衡(g/kg/day)係數的平均值±標準差,分別是 LenP 組為 81±6及 0.35±0.21,AP 組為 82±5及 0.36±0.08, 而 LP 組為 73±4及 0.13±0.06 (全部氮吸收及平衡比較:LenP vs. AP,p>0.05; LenP vs. LP,p<0.05; AP vs. LP,p<0.05)。此結果顯示不論是扁豆或是動物性來源的高蛋白飲食均有較高的氮吸收及氮平衡,這可能促進組織蛋白質儲存。以扁豆為主的高蛋白飲食較便宜,適用於中度營養不良的兒童之營養性修復。

關鍵字:扁豆為主的高蛋白飲食、動物來源高蛋白飲食、氮吸收、氮平衡、營 養不良