Effect of varying protein and energy intakes on nitrogen balance in Indian preschool children

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1. The effect of varying protein intake at two energy levels of 334 and 418 kJ/kg body-weight was studied in four preschool children belonging to the low socio-economic group.

2. Results indicated a curvilinear relationship between N intake and N retention. From this relationship, the protein requirement of the children at adequate energy intake was calculated.

3. At adequate energy intake (418 kJ/kg body-weight) the protein requirement of the children was 1·33 g/kg. On decreasing the energy intake by 20 % to 334 kJ/kg body-weight the protein requirement was found to be increased by 20 % to 1·64 g/kg.

4. Based on this study, a safe level of protein intake for Indian preschool children subsisting on a diet based predominantly on vegetable proteins has been suggested.

Protein-energy malnutrition is an important nutritional problem among preschool children in many developing countries of the world. In order to define the extent of this problem it is essential to have a precise knowledge of the protein and energy requirements of preschool children. The Expert Group of the FAO/WHO (Joint FAO/WHO Expert Group on Protein Requirements, 1965) derived the protein requirements of preschool children by the factorial method and suggested safe levels of intake; these values were subsequently revised (Joint FAO/WHO Expert Committee on Energy and Protein Requirements, 1973). The latter levels have been adopted by other countries with modifications to suit their local conditions. The Nutrition Expert Committee of the Indian Council of Medical Research (Gopalan & Narasinga Rao, 1968) recommended a protein intake of 2 g/kg body-weight, the protein being generally derived from plant sources, and an energy intake of 418 kJ (100 kcal)/kg body-weight for preschool children. The safe level of protein recommended for preschool children presumes that energy intake is adequately met. However, results of several carefully-conducted diet surveys have shown that the energy intake of a large proportion of children belonging to the low socio-economic group is below the recommended level of 418 kJ/kg body-weight, although their protein intake corresponds to the safe allowances (Narasinga Rao *et al.* 1969). The mean deficit in energy intake in most of the children belonging to the age-group 3–4 years has been found to be approximately 20 %, ranging from 13 to 40 % (Indian Council of Medical Research, 1974). A question of practical importance is whether their current levels of protein intake would meet their requirements when energy needs are not fully met. In order to determine protein requirements of such children in relation to energy intake a nitrogen balance study was carried out, varying their protein intake at two levels of energy intake, one corresponding to the recommended level of energy intake (418 kJ/kg body-weight) and the other corresponding to their actual energy intake (334 kJ/kg body-weight).

MATERIALS AND METHODS

Four healthy preschool children belonging to the low socio-economic group among whom energy deficiency is widely prevalent were admitted to the Nutrition Unit of the Niloufer hospital. These children were under the constant supervision of a nurse throughout the study. Relevant details of the subjects are given in Table 1. Their heights and weights,

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Table 1. *Anthropometric measurements for four Indian preschool children*

Subject	Sex	Age (years)	Height (m)	Wt (kg)	Wt-for-height (%) Indian*	Wt-for-height (%) Standard†	Wt-for-age (%) Indian*	Wt-for-age (%) Standard†	Height-for-age (%) Indian*	Height-for-age (%) Standard†
GE	♂	4·0	0·980	13·6	101	92	101	82	102	74
SKA	♀	4·5	0·910	12·8	105	95	99	74	96	85
SK	♂	3·5	0·850	9·8	87	82	83	63	99	85
FR	♀	4·5	1·010	13·6	94	86	105	78	107	95

* Calculated from results of the Indian Council of Medical Research (1972).
† Calculated from results of Jelliffe (1966).

Table 2. *Composition (g) of diets fed to Indian preschool children**

Energy level (kJ/kg body-wt) ...	334				418			
Protein level (g/kg body-wt) ...	2·0	1·6	1·3	1·0	2·0	1·6	1·3	1·0
Ingredient								
Rice	12·0	10·0	10·0	10·0	12·0	10·0	10·0	10·0
Red gram (*Cajanus cajan*)	1·5	1·0	0·5	0·5	1·5	1·0	0·5	0·5
Green gram (*Phaseolus radiatus*)	1·5	0·5	0·5	—	1·5	0·5	0·5	—
Vegetables	5·0	5·0	5·0	2·5	5·0	5·0	5·0	2·5
Milk	10·0	10·0	5·0	3·0	10·0	10·0	5·0	3·0
Sugar	2·0	3·0	3·0	3·0	3·0	3·0	3·0	3·0
Cassava (*Manihot esculenta*) starch	—	1·8	2·0	3·0	2·8	5·7	6·9	7·9
Oil	1·5	1·5	2·0	2·0	2·0	2·0	2·0	2·0

* For details, see Table 1.

although comparable to the standards for Indian children (Indian Council of Medical Research, 1972) were only 74–95% for height and 63–82% for weight as compared to International Standards (Jelliffe, 1966). Although they were in the hospital, the children were not confined to bed except at night during sleep and their normal activity was not restricted. Heights and weights of all subjects were recorded before the commencement of the experiment and weights re-recorded at the beginning of each dietary regimen. The experimental diet was formulated so as to resemble the habitual diet consumed at home, which was determined by an oral questionnaire method. Their diets consisted mainly of rice and small quantities of legumes, vegetables, milk, sugar and fat.

Experimental diet

The subjects were fed at two energy levels, i.e. the habitual intake of 334 kJ/kg body-weight and the recommended level of 418 kJ/kg body-weight; and four protein levels: 1·0, 1·3, 1·6, and 2·0 g/kg body-weight. The compositions of the diets are given in Table 2.

Experimental design

Each of the subjects received diets on all four protein levels on a Latin square design. They were fed first at 334 kJ/kg body-weight, and then at 418 kJ/kg body-weight. Each diet was given for a period of 11 d. Urine and faeces (24 h samples) were collected during the last 4 d of each dietary regimen. Urine was collected in toluene and acetic acid, while faeces were collected in plastic containers. Urine and faeces were analysed for total N. Urine was also analysed for creatinine.

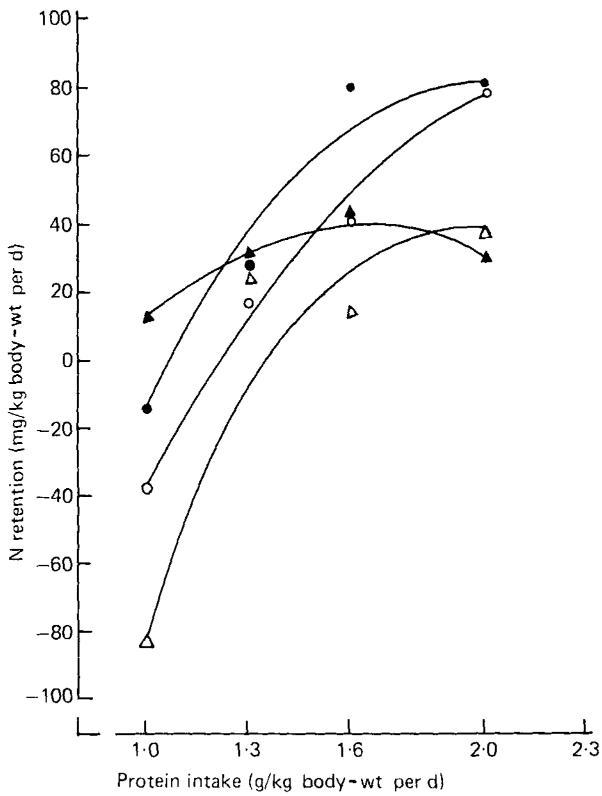

Fig. 1. Nitrogen balance in Indian preschool children given varying levels of protein (energy level, 334 kJ/kg body-wt). ○——○, GE; ●——●, SKA; △——△, SK; ▲——▲, FR. For details of diets, see Table 2.

Analytical methods

Daily faecal collections were mixed thoroughly in a Waring blender and portions analysed in duplicate for N. Urine collections (24 h) were mixed well, volume measured and portions taken for analysis. Total N in urine and faeces was determined by the macrokjeldhal procedure. Urinary creatinine was determined by the method of Clark & Thompson (1949) using urine which had been diluted tenfold.

RESULTS AND DISCUSSION

Results for N intake, faecal N, urinary N, creatinine and N balance are presented in Table 3.

N losses from other sources, e.g. skin, were not determined in this study. Based on values provided by the FAO/WHO Expert Committee on Protein and Energy requirements (1973) cutaneous losses were assumed to be approximately 0·48 mg N/basal kJ or 10 mg N/kg.

N balance was found to be positive in all subjects fed at the following energy (kJ) and protein (g) levels (/kg body-weight): 334, 2; 334, 1·6; 334, 1·3. However at 334 kJ and 1·0 g protein/kg body-weight one subject (FR) showed only a marginal positive N balance while the other three subjects went into negative N balance. At the following energy (kJ) and protein (g) levels (/kg body-weight): 418, 2; 418, 1·6; 418, 1·3, all the children were in positive balance, while at 418 kJ and 1·0 g protein/kg body-weight three of the four subjects were in positive balance with only one subject (SK) showing a marginally-negative balance.

Table 3. *Nitrogen balance in Indian preschool children* given varying levels of protein*

(Mean values with their standard errors)

Dietary level (/kg body-wt) of: Energy (kJ)	Protein (g)	Subject	N intake (g)	Urinary N (g) Mean	Urinary N (g) SE	Faecal N (g) Mean	Faecal N (g) SE	N excreted (g)	Balance	N retention (mg/kg body-wt)	Creatinine (mg/24 h) Mean	Creatinine (mg/24 h) SE
334	2	GE	4·35	1·84	0·070	1·44	0·100	3·28	+1·07	+78·7	179·3	6·43
		SKA	4·16	1·93	0·077	1·18	0·049	3·11	+1·05	+80·8	166·9	3·58
		SK	3·07	1·74	0·085	0·97	0·120	2·71	+0·36	+37·5	123·8	5·87
		FR	4·35	2·61	0·066	1·31	0·153	3·94	+0·41	+30·1	152·8	9·25
		Mean	3·98	2·03		1·23		3·26	+0·72	+56·8		
334	1·6	GE	3·58	1·60	0·032	1·41	0·085	3·01	+0·57	+40·7	187·4	1·64
		SKA	3·28	1·25	0·098	1·00	0·166	2·25	+1·03	+80·5	153·1	13·24
		SK	2·46	1·48	0·013	0·83	0·335	2·31	+0·15	+15·6	119·1	5·13
		FR	3·46	1·74	0·012	1·14	0·015	2·88	+0·58	+43·0	161·1	6·21
		Mean	3·20	1·52		1·10		2·62	+0·58	+44·9		
334	1·3	GE	2·91	1·55	0·026	1·12	0·035	2·67	+0·24	+17·1	171·0	5·40
		SKA	2·74	1·38	0·056	1·00	0·325	2·38	+0·36	+27·3	159·9	2·53
		SK	2·04	1·02	0·007	0·74	0·042	1·76	+0·28	+28·6	113·2	9·07
		FR	2·79	1·24	0·082	1·12	0·125	2·36	+0·43	+32·1	150·9	1·63
		Mean	2·62	1·30		1·00		2·30	+0·32	+26·3		
334	1·0	GE	2·18	1·52	0·072	1·17	0·067	2·69	−0·51	−37·5	171·6	13·23
		SKA	2·02	1·26	0·059	0·94	0·335	2·20	−0·18	−14·3	166·9	7·93
		SK	1·50	1·66	0·009	0·62	0·055	2·28	−0·78	−83·0	123·9	2·11
		FR	2·18	1·02	0·074	1·00	0·270	2·02	+0·16	+11·8	127·5	11·30
		Mean	1·97	1·37		0·93		2·30	−0·33	−30·8		
418	2	GE	4·35	2·01	0·088	1·23	0·278	3·24	+1·11	+81·6	183·1	3·34
		SK	3·20	1·80	0·064	0·97	0·135	2·77	+0·43	+43·6	139·5	7·14
		FR	4·67	2·11	0·078	1·06	0·398	3·17	+1·50	+102·7	155·6	9·84
		Mean	4·07	1·97		1·09		3·06	+1·01	+75·8		
418	1·6	GE	3·68	1·30	0·057	1·32	0·149	2·62	+1·06	+73·6	158·7	9·20
		SK	2·66	1·32	0·054	0·88	0·223	2·20	+0·46	+44·2	122·8	5·90
		FR	3·66	1·06	0·104	1·32	0·413	2·44	+1·22	+85·3	151·3	11·72
		Mean	3·33	1·23		1·19		2·42	+0·91	+67·7		
418	1·3	GE	2·99	1·01	0·101	1·27	0·073	2·28	+0·71	+49·3	165·7	3·32
		SK	2·00	0·98	0·064	0·82	0·284	1·80	+0·20	+20·8	113·8	7·39
		FR	2·91	1·07	0·062	1·31	0·160	2·38	+0·53	+37·8	154·2	6·42
		Mean	2·63	1·02		1·13		2·15	+0·48	+35·9		
418	1·0	GE	2·33	0·98	0·042	1·08	0·168	2·06	+0·27	+18·5	159·4	9·32
		SK	1·63	1·05	0·063	0·67	0·020	1·72	−0·09	−8·8	126·6	8·30
		FR	2·18	1·10	0·029	1·04	0·203	2·14	+0·04	+2·9	139·7	12·20
		Mean	2·05	1·04		0·93		1·97	+0·08	+4·2		

* For details, see Table 1.

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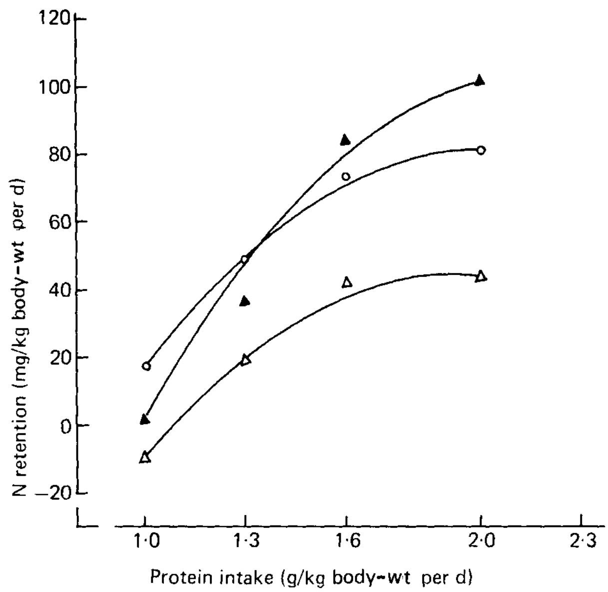

Fig. 2. Nitrogen balance in Indian preschool children given varying levels of protein (energy level, 418 kJ/kg body-wt). ○——○, GE; △——△, SK; ▲——▲, FR. For details of diets, see Table 2.

Table 4. *Regression equations at different energy intakes for Indian preschool children*

Subject	Energy level (kJ/kg body-wt)	Type of regression	Regression equation	r^2
	334	Linear		
GE			$y = -140{\cdot}19+0{\cdot}69x$	0·9644
SKA			$y = -103{\cdot}24+0{\cdot}62x$	0·8522
SK			$y = -151{\cdot}35+0{\cdot}64x$	0·6134
FR			$y = -2{\cdot}4+0{\cdot}11x$	0·3600
	334	Quadratic		
GE			$y = -264{\cdot}56+1{\cdot}79x-2{\cdot}28x^2$	0·9889
SKA			$y = -358{\cdot}81+2{\cdot}88x-4{\cdot}68x^2$	0·9676
SK			$y = -472{\cdot}92+3{\cdot}48x-5{\cdot}92x^2$	0·8482
FR			$y = -165{\cdot}59+1{\cdot}59x-3{\cdot}08x^2$	0·9914
	418	Linear		
GE			$y = -37{\cdot}53+0{\cdot}39x$	0·9063
SK			$y = -53{\cdot}05+0{\cdot}33x$	0·8166
FR			$y = -95{\cdot}27+0{\cdot}64x$	0·9455
	418	Quadratic		
GE			$y = -177{\cdot}78+1{\cdot}63x-2{\cdot}57x^2$	0·9977
SK			$y = -224{\cdot}60+1{\cdot}84x-3{\cdot}14x^2$	0·9930
FR			$y = -236{\cdot}46+1{\cdot}89x-2{\cdot}59x^2$	0·9818

At each corresponding level of N intake, N retention at the 418 kJ/kg level was higher than that at an intake of 334 kJ/kg (Table 3). Also, for a given N retention, protein intake was higher at the 334 kJ/kg level than at 418 kJ/kg.

The relationship between N intake and N balance was tested statistically to determine the 'best fit' by a linear regression ($y = a+bx$) and by a curvilinear regression ($y = a+bx+cx^2$), where x and y are the N intake (mg/kg body-weight per d) and N retention (mg/kg body-weight per d) respectively, and a, b, c are constants. The regression equations for each

Table 5. *Protein requirements (g/kg body-weight) at different energy intakes for Indian preschool children*

Subject	Energy level (kJ/kg body-wt)	Type of regression	Protein requirement for N equilibrium (g)	Protein requirement for N retention of 40 mg/kg body-wt
	334	Quadratic		
GE			1·22	1·54
SKA			1·08	1·32
SK			1·32	1·91
FR			0·89	1·77
Mean			1·13	1·64
SD			0·186	0·259
95% confidence limits of mean			0·76–1·50	1·12–2·16
	418	Quadratic		
GE			0·87	1·19
SK			1·07	1·55
FR			1·00	1·26
Mean			0·98	1·33
SD			0·101	0·191
95% confidence limits of mean			0·78–1·18	0·95–1·71

individual at two different energy levels were determined by the 'least squares' method. Table 4 gives the linear and quadratic equations in each instance, together with values for coefficients of determination (r^2). The r^2 value for the quadratic equation was higher than that for the linear equation in each instance, the difference being in the majority highly significant. On the basis of this finding the quadratic equation was accepted as a better means of describing the relationship between N intake and N retention.

The mean intake for N equilibrium and for a retention of 40 mg N/kg body-weight per d at both energy levels, calculated from the quadratic equation are given in Table 5.

It has been suggested that a positive retention of 40 mg N/kg body-weight per d (De Maeyer & Vanderborght, 1961) would meet N requirements for growth, cutaneous and other losses of N in most individuals, and also the possible over-estimates inherent in N balance determinations. Among the preschool children participating in the present study an intake of $1·33 \pm 0·191$ g protein/kg body-weight was necessary for an N retention of 40 mg/kg body-weight at 418 kJ/kg body-weight. This would correspond to a safe intake of 1·71 g protein/kg body-weight per d. Thus a diet providing 70 g protein-energy/kg and containing mixed proteins of the type used in the present study would meet their protein requirement provided their energy requirements were met.

Using 40 mg N retention/kg as a measure of adequacy, the protein requirement of the children studied would be 1·64 g/kg body-weight at an energy intake of 334 kJ/kg body-weight as compared to 1·33 g/kg body-weight at 418 kJ/kg body-weight. This corresponds to a 20% increase in the protein requirement at the lower energy intake. Similarly the protein intake (1·13 g/kg body-weight) to maintain these children at N equilibrium would be increased by 10% at the lower energy level (334 kJ/kg body-weight) as compared to the intake (0·98 g/kg) at the higher energy level (418 kJ/kg body-weight).

Results of similar studies in adult men reported earlier (Iyengar & Narasinga Rao, 1979) have also shown that protein intake for N equilibrium would be higher as the result of a decrease in energy intake. However, a comparison of results of the earlier study and those of the present study shows that the increase in protein intake to meet the N equilibrium criteria when the energy intake is lowered by 20%, is of a lower magnitude in children than in adults.

Results presented here show that safe levels of protein intake can be achieved on diets which contain 70 g protein-energy/kg when energy intake is 418 kJ/kg body-weight. At lower energy levels of 334 kJ/kg body-weight, the diet would have to provide 90–100 g of protein-energy/kg to achieve a positive N retention of 40 mg/kg body-weight per d. Results of dietary surveys indicate that the habitual diets of preschool children in India provide approximately 110–120 g protein-energy/kg (Narasinga Rao *et al.* 1969). The habitual diets thus provide adequate protein intake even if the energy intake is 20% below requirement. However, protein requirements on the habitual diets may not be met if the energy deficit exeeds 20%.

The children in the present study belonged to the low socio-economic group and were undernourished as judged by anthropometric criteria. Their body-weights were 75% and heights 85% of the International Standards (Jelliffe, 1966). Their creatinine levels were also lower as compared to well-nourished Western children, suggesting a deficit in their muscle mass also. However the majority of Indian children belong to this latter category. The results obtained for protein requirements in this study would therefore be particularly applicable to Indian preschool children.

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