Nitrogen balance in Indian preschool children receiving the safe level of protein at varying levels of energy

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1. A study was carried out to determine the effects of varying the level of energy intake on nitrogen balance in preschool children receiving the safe requirement level of protein, determined in an earlier study.

2. Seven preschool children received four energy levels, i.e. 293, 334, 376 and 418 kJ/kg body-weight at the safe level of protein intake of 1·75 g/kg body-weight and N balance determined.

3. The N balance decreased with a decrease in energy intake. However, the N balance was positive at all levels of energy intake studied.

4. Results indicated that at a protein intake of 1·75 g/kg body-weight the minimum level of energy intake for a retention of 40 mg N/kg body-weight in these children was found to be 326·2±45·5 (mean±SD) kJ/kg body-weight. Below this energy intake the safe level of protein intake became inadequate.

Protein-energy malnutrition is a major nutritional problem among preschool children in many developing countries of the world. In order to define the extent of this problem, one must have a precise knowledge of their protein and energy requirements. Results of several carefully-conducted diet surveys among Indian preschool children belonging to low-income groups subsisting on cereal–pulse diets have shown that their protein intakes correspond to currently recommended levels, while their energy intakes fail to meet their requirement (Narasinga Rao *et al.* 1969; Gopalan & Narasinga Rao, 1971), and the average energy deficit is approximately 30%. It is possible that in the face of this energy deficit their current protein intakes may not be adequate. However, not much information is available regarding protein requirements in Indian preschool children in relation to their energy intakes.

In a previous communication, Iyengar *et al.* (1979) reported that the safe level of protein intake for Indian preschool children is 1·75 g/kg body-weight provided the energy intakes are adequate at 418 kJ/kg body-weight. It is not clear however, whether this level of protein intake would be adequate in the face of inadequate energy intake usually observed among those children belonging to the low socio-economic group. Available information suggests that inadequate energy intake decreases protein utilization (Munro, 1951). It has been well documented that in adult men, at an adequate level of protein intake, the energy level becomes the deciding factor for N balance (Calloway & Spector, 1954; Nageswara Rao *et al.* 1975). It was therefore considered important to determine the influence of variation in energy intakes on N metabolism in preschool children receiving the safe level of protein. The present study was also aimed at determining the minimal energy intake below which the safe level of protein intake would become clearly inadequate to meet the protein needs of preschool children. Such information would be helpful in assessing the extent of protein deficiency in children with different extents of energy inadequacy.

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Table 1. *Anthropometric measurements of Indian preschool children studied*

Subject	Sex	Age (years)	Weight (kg)	Height (m)	% Weight-for-age *a*	% Weight-for-age *b*	% Height-for-age *a*	% Height-for-age *b*	% Weight-for-height *a*	% Weight-for-height *b*
PS	♂	4·0	12·6	0·934	76	93	90	97	91	102
JP	♂	3·5	11·4	0·932	74	97	94	105	81	92
NS	♀	3·0	11·2	0·923	80	95	96	98	82	92
RA	♂	4·0	14·0	0·985	85	108	95	102	93	103
NR	♂	3·5	12·4	0·875	80	92	87	91	100	108
NJ	♀	4·0	14·0	0·954	85	108	92	101	98	106
RU	♂	3·0	10·3	0·820	71	87	86	92	90	100

a, Calculated from Jelliffe (1966); *b*, calculated from the Indian Council of Medical Research (1972).

Table 2. *Composition of diets (g/kg body-weight) fed to Indian preschool children*

	Energy levels (kJ) 293	334	376	418
Rice	12·0	12·0	12·0	12·0
Red gram (*Cajanus cajan*)	1·0	1·0	1·0	1·0
Green gram (*Phaseolus radiatus*)	1·0	1·0	1·0	1·0
Potatoes	5·0	5·0	5·0	5·0
Brinjals (*Solanum melongena*)	5·0	5·0	5·0	5·0
Milk	10·0	10·0	10·0	10·0
Cassava starch (*Manihot esculenta*)	0·5	0·5	0·5	0·5
Sugar	1·0	2·0	4·0	4·0
Refined groundnut oil	0·3	1·0	1·3	2·3

MATERIALS AND METHODS

Seven apparently-normal preschool children were admitted to the Nutrition Ward of the Niloufer Hospital, Hyderabad, India, and were under the supervision of a nurse throughout the study. Relevant details of the subjects are given in Table 1. Their heights and weights, although comparable to the standards for Indian children (Indian Council of Medical Research, 1972), were only 71–85% for weight and 86–96% for height as compared to International standards (Jelliffe, 1966). Although they were in the hospital, the children were not confined to bed except during sleep at night and their normal activities were not curtailed. Heights and weights of all subjects were recorded before the commencement of the experiment and weights were recorded daily. The experimental diet was formulated so as to correspond closely to their habitual diet which was determined by an oral questionnaire method. Their diets comprised mainly rice and small quantities of legumes, vegetables, milk, sugar and fat.

Experimental diets

The subjects received diets containing a protein level of 1·75 g/kg body-weight and four levels of energy intake i.e. 293, 334, 376 and 418 kJ/kg body-weight (70, 80, 90 and 100 kcal/kg body-weight) respectively. The composition of the diets is given in Table 2. The

Table 3. *Nitrogen balance in Indian preschool children receiving varying levels of energy*

(Mean values with their standard errors)

Energy intake (kJ/kg body-wt)	Subject	Body-wt (kg)	Diet period	Urinary N (g/24 h)		Faecal N (g/24 h)		Creatinine (mg/24 h)		N balance	
				Mean	SE	Mean	SE	Mean	SE	(g/d)	(mg/kg body-wt per d)
293	PS	12·6	4	1·74	0·088	0·81	0·106	148	5·4	0·98	77·7
	JP	11·6	2	1·96	0·146	0·92	0·274	145	5·2	0·37	31·9
	NS	11·4	3	1·81	0·034	1·02	0·195	112	6·6	0·36	31·5
	RA	14·0	4	2·66	0·089	0·92	0·120	171	4·5	0·34	24·3
	NR	12·4	2	2·07	0·079	0·86	0·055	149	8·7	0·54	43·5
	NJ	14·1	3	2·84	0·081	0·72	0·105	167	10·9	0·39	27·6
	RU	10·6	1	1·91	0·046	0·67	0·106	142	5·8	0·39	36·8
	Mean			2·14		0·85		148		0·48	39·0
	SE			0·163		0·046		7·3		0·087	6·86
334	PS	12·8	3	1·69	0·109	0·89	0·121	156	16·7	1·0	79·4
	JP	11·4	4	1·76	0·163	1·15	0·263	124	7·6	0·28	24·6
	NS	11·4	1	1·87	0·046	0·97	0·247	116	4·8	0·35	30·7
	RA	14·0	3	2·56	0·106	0·98	0·052	178	5·1	0·38	27·1
	NR	12·4	4	1·80	0·063	0·94	0·100	149	6·8	0·68	55·7
	NJ	14·3	1	2·49	0·119	0·80	0·095	182	6·6	0·71	49·6
	RU	10·6	2	1·62	0·095	0·78	0·176	140	4·9	0·57	53·7
	Mean			1·97		0·93		149		0·57	45·8
	SE			0·147		0·047		9·48		0·096	7·45
376	PS	12·6	2	1·54	0·122	0·75	0·009	162	7·9	1·24	98·4
	JP	11·3	1	1·61	0·139	1·08	0·058	141	10·1	0·47	41·6
	NS	11·2	4	1·62	0·060	0·88	0·193	120	8·5	0·64	57·1
	RA	14·2	2	2·37	0·083	1·05	0·147	178	5·9	0·56	39·4
	NR	12·6	1	1·67	0·105	1·01	0·590	165	5·3	0·76	60·3
	NJ	14·0	4	2·31	0·083	0·85	0·104	195	3·8	0·76	54·3
	RU	10·4	3	1·50	0·090	0·91	0·029	129	3·9	0·50	48·1
	Mean			1·80		0·93		156		0·70	57·0
	SE			0·140		0·045		10·2		0·099	7·49
418	PS	12·6	1	1·52	0·111	0·79	0·215	157	5·4	1·22	96·8
	JP	11·4	2	1·54	0·185	1·13	0·100	142	9·1	0·52	45·6
	NS	11·2	3	1·55	0·076	0·94	0·029	109	7·2	0·65	58·0
	RA	14·3	1	2·13	0·088	0·92	0·094	179	3·0	0·95	67·8
	NR	12·2	2	1·49	0·052	0·89	0·071	166	4·9	1·04	85·2
	NJ	14·3	3	2·04	0·120	0·81	0·072	190	8·2	1·15	80·4
	RU	10·3	4	1·23	0·096	0·82	0·047	141	7·1	0·83	80·6
	Mean			1·64		0·090		155		0·91	73·5
	SE			0·122		0·044		10·2		0·098	6·60

Table 4. *ANOVA for data on nitrogen balances and energy levels*

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F ratio
Between energy levels	3	0·729011	0·2430	20·9*
Between individuals	6	1·304571	0·2174	18·7*
Error	18	0·209514	0·0116	—
Total	27	2·243096	0·4720	—

* $P < 0·001$.

cereal and pulse contents of the diets were kept constant while the sugar and fat contents were varied to achieve different energy levels.

Experimental design

Each of the subjects received diets at all four energy levels, each level being fed for a period of 11 d. Urine and faeces (24 h samples in each instance) were collected during the last 4 d of each dietary regimen. Urine was collected under toluene and acetic acid and stored in the cold for subsequent analyses, while faeces were collected in suitable plastic containers. Urine and faeces were analysed for total N. Urine was also analysed for creatinine.

Analytical methods

Urine and faeces were analysed for total N by the macrokjeldhal method. Urinary creatinine was estimated by the method of Clark & Thompson (1949) using tenfold dilution of urine.

RESULTS AND DISCUSSION

Values for N intake, faecal N, urinary N, N balance and creatinine excretion for individual subjects are presented in Table 3. Statistical analysis of the results was carried out by the two-way analysis of variance and the statistical significance was tested by the *t* test. The results of analysis are given in Table 4.

N balance was positive in all the subjects on all four levels of energy intake studied. N retention tended to decrease as the energy decreased. However no meaningful mathematical relationship could be obtained between energy intake and N retention, although statistical analysis of N retention values in relation to energy intake revealed that N retention at an energy intake of 418 kJ/kg body-weight was significantly ($P < 0·01$) higher when compared to N retention observed on intakes of 376 kJ, 334 kJ or 293 kJ/kg body-weight. N retention at an energy intake of 376 kJ/kg body-weight was significantly ($P < 0·05$) higher than at intakes of either 334 kJ or 293 kJ and N retentions at the latter two levels were not significantly different from each other. The increased N retention with increasing energy intake reported in the present study suggests a protein sparing action of energy, which has also been observed by several earlier workers (Plough *et al.* 1956; Inoue *et al.* 1973; Nageswara Rao *et al.* 1975). Munro & Naismith (1953) showed in rats that even when the diet contained no protein, the addition of energy as carbohydrate produced a small improvement in N balance.

Urinary N excretion increased as the level of energy intake was decreased, reflecting the changes in N retention. However, neither faecal N excretion (g/d) nor urinary creatinine-N excretion (g/d) was affected by the level of energy intake. There was a significant inverse linear relationship between energy intake (X; kJ/kg body-weight per d) and urinary N excretion (Y; g/kg body-weight per d) in each of the subjects (Table 5).

Table 5. *Relationship between energy intake and urinary nitrogen excretion in Indian preschool children receiving the safe level of protein*

Subject	Regression equation, where X is energy intake (kJ/kg body-wt per d) and Y is urinary N (g/kg body-wt per d)	SE	Correlation coefficient	Improvement in N balance (mg N/kJ)	Energy intake (kJ/kg body-wt) for N retention of 40 mg/kg body-wt
PS	$Y = 0{\cdot}1837 - 0{\cdot}000153\,X$	0·00003719	−0·9496	0·153	248·0
JP	$Y = 0{\cdot}2595 - 0{\cdot}000304\,X$	0·00005707	−0·9682	0·304	390·6
NS	$Y = 0{\cdot}2346 - 0{\cdot}000227\,X$	0·00009333	−0·8606	0·277	335·0
RA	$Y = 0{\cdot}2752 - 0{\cdot}000282\,X$	0·00001872	−0·9878	0·282	358·9
NR	$Y = 0{\cdot}2699 - 0{\cdot}000360\,X$	0·00005641	−0·9776	0·360	294·7
NJ	$Y = 0{\cdot}3266 - 0{\cdot}000542\,X$	0·00005184	−0·9926	0·542	328·1
RV	$Y = 0{\cdot}3483 - 0{\cdot}000542\,X$	0·00005184	−0·9926	0·542	328·1
Average of regressions	$Y = 0{\cdot}2711 - 0{\cdot}000344\,X$			0·344	Median 328·1

From the relationship between urinary N and energy intake, the mean improvement in N balance was found to be 0·34 mg N/kJ (1·44 mg N/kcal).

The present study indicates that in children receiving a safe level of protein, a decrease in energy intake of 30%, i.e. from 418 to 293 kJ/kg body-weight did not result in negative N balance, although the retention had decreased significantly. An intake of 293 kJ/kg body-weight in these children represents approximately their maintenance requirement, i.e. 1·5 × basal requirement (Joint FAO/WHO Expert Committee on Energy and Protein Requirements, 1973). In contrast, in adults, an energy intake below the maintenance level resulted in negative N balance (Nageswara Rao *et al.* 1975). These observations suggest that there are differences between adults and children in their response to alterations in energy intake in N metabolism.

The level of energy intake below which the safe level of protein intake of 1·75 g/kg body-weight becomes inadequate can be computed from the present information. In arriving at a safe level of protein requirement of these children, a retention of 40 mg N/kg body-weight was considered to be adequate to meet their growth requirements (Iyengar *et al.* 1979). From the previously-mentioned relationship between energy intake and urinary N loss and the observed faecal N loss (which was not related to energy intake), the minimum energy intake for a retention of 40 mg N/kg body-weight was calculated and the values are given in Table 4. It can be seen that the minimum energy intake (mean ± SD) in these children for an N retention of 40 mg/kg body-weight was found to be 326 ± 46 kJ/kg body-weight.

The influence of energy intake on the efficiency of protein utilization is well documented (Allison, 1958). The results presented here provide further support to this. Net protein utilization (NPU) of dietary protein in these children was calculated from the N retained and the endogenous N loss, which was assumed to be 2 mg N/basal energy. The NPU decreases from 0·60 at an energy intake of 418 kJ/kg body-weight to 0·47 at an energy intake of 293 kJ/kg body-weight. Thus, a decrease in energy intake of 30% i.e. from 418 to 293 kJ/kg body-weight resulted in a similar extent of decrease in NPU.

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