Human protein requirements: obligatory urinary and faecal nitrogen losses and the factorial estimation of protein needs of Nigerian male adults

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1. The present study was designed to use the factorial approach to estimate protein requirements of Nigerian male adults by measuring obligatory nitrogen losses via urine, faeces and sweat when N intake was very low and energy intake adequate.

2. Eight adult men from Osegere village near Ibadan and seven medical students from the University of Ibadan, who volunteered to participate as subjects in the study, were given a low-protein diet (based on staple foods habitually consumed by subjects) for 10 d. Mean daily total protein intake was 4·68 g while that of energy was 0·2 MJ/kg body-weight. After an initial 5 d adaptation period, 24 h urine and faeces were collected in marked containers for five consecutive days for N determination. N losses from the skin were also determined in the village adults.

3. Mean daily urinary, faecal and sweat N losses (mg N/kg body-weight) were 45·88 (SD 4·84), 21·79 (SD 4·19), and 7·46 (SD 1·71) from the village adults. The corresponding urinary and faecal N losses from the university students were 43·45 (SD 2·28) and 18·32 (SD 4·66) (sweat N loss not measured). Thus the total daily obligatory N losses (per kg body-weight) from the village adults and university students were 75·13 and 69·23 mg N respectively (assuming a sweat N loss of 7·46 mg for the university students). After adjusting for requirement and making a 30% allowance for individual variability, the safe level of protein intake was calculated to be 0·78 and 0·73 g protein/kg body-weight for the village men and university students respectively.

4. These values suggest that the Joint FAO/WHO *ad hoc* Expert Committee (1973) safe allowance of 0·57 g egg protein/kg per d is not sufficient for most young adults in this environment when energy intake is adequate.

The Joint FAO/WHO *ad hoc* Expert Committee (FAO/WHO) (1973) used the factorial method to estimate the safe protein intake for healthy, well-nourished Caucasian males and arrived at a value of 0·57 g egg protein/kg body-weight as sufficient to meet the needs of nearly all (97·5%) normal adult Caucasians consuming generous energy intakes. Calculation of protein requirements by the factorial method assumes that the total urinary and faecal nitrogen losses, determined after adaptation to an essentially N-free diet (endogenous urinary and faecal N), plus integumental and other miscellaneous losses, equate with physiological requirements for dietary protein of high biological value. In this respect, a correction factor of 1·3 must be made for the efficiency of dietary N utilization at requirement levels of intake in addition to another allowance (factor of 1·3) for individual variation so that the needs of 97·5% of the population are covered.

While accepting the factorial method adjusted by a factor considered to be appropriate, the FAO/WHO (1973) report emphasized the need for comparative studies of endogenous N losses in different ethnic groups. Scrimshaw *et al.* (1972) considered it pertinent to ask whether the results obtained with Caucasian university students apply equally to population groups of different racial origins and nutritional backgrounds. Indeed, Huang *et al.* (1972) suggested that Chinese men may be able to maintain N balance at lower levels of N intake, expressed on a body-weight or basal metabolic rate basis, than similar subjects of Caucasian origin. Furthermore, earlier studies carried out in Nigeria (Nicol & Phillips, 1976*a*, *b*) suggest that there may be significant ethnic differences in protein requirements, and that

populations of developing countries may be more adapted genetically or phenotypically to lower protein intakes than are Caucasians.

The purpose of this paper is twofold: (1) to reassess values for endogenous N losses in Nigerian men selected from a low-income group and a group of university students and (2), to estimate the protein requirements of the groups using the obligatory urinary and faecal losses obtained.

MATERIALS AND METHODS

Subjects

Eight village adult men were recruited into the study along with seven medical students from the University of Ibadan. The village men (VO1–VO8) were aged between 30 and 40 years while the university students (HO1–HO7) were between 19 and 23 years old. It was not possible to recruit younger subjects in the village as this age-group had migrated to the urban centres.

The village adult males were farmers of small holdings whose health was evaluated by medical history, physical examination and routine blood chemistry before they were approved for the study. They had all lived in Osegere village, an experimental village for the Department of Human Nutrition, for the past 20 years. The department runs a rural health clinic in this village (about 20 km from the university). All the village subjects lived in the rural health clinic throughout the duration of the study which lasted for 13 d, and were under close medical supervision. During the study they were allowed to maintain a minimum constant level of physical activity such as playing indoor games, light cleaning and story telling. Their mean weight at the start of the study was 50·49 (SD 5·29) kg.

The university students were all third-year medical students living in dormitories close to the teaching hospital compound. They were studied on an outpatient basis but under close medical supervision. During the study they were engaged in their routine daily activities while maintaining a reasonable constant level of physical activity which was closely monitored. They attended their normal classes and refrained from any strenuous exercises. All subjects were fully informed of the purpose, nature and design of the experiment and all gave informed consent. The design of the experiment was approved by the College of Medicine Ethical Committee. Table 1 summarizes the characteristics of the subjects.

Diets

The average composition of the low-protein diet is shown in Table 2. Energy intake to provide energy equilibrium was calculated from a dietary history interview and maintained without change during the study. The experimental diet was standardized, based on staple foods usually consumed by the subjects and served in three approximately isoenergetic meals at 08.00, 13.00 and 19.00 hours respectively. The breakfast consisted of custard or maize starch with sugar while the lunch and dinner were made up of cassava (*Manihot esculenta*) meal (Gari) with stew. Caloreen (a glucose polymer) and orange drinks were used to raise the energy intake to the desired level. The meals were prepared according to the dietician's recipe and consumed under her supervision.

Experimental design and collection of samples

After a 3 d free choice diet period, the subjects were given the 'low-protein' diet for 10 d. The first 5 d were for the adaptation period while the remaining 5 d were for metabolic study. However, daily urine collection was undertaken throughout the 10 d period to determine the daily urinary N excretion patterns. Carmine dye capsules (0·5 g) were given to serve as a faecal marker to indicate the beginning and end of the 5 d faecal collection period.

Table 1. *Characteristics of subjects*

Subjects	Age (years)	Height (m)	Wt (kg)	Mid-arm circumference (mm)	Tricep skinfold (mm)
Village					
VO1	37	1·55	50·9	253·2	4·20
VO2	39	1·68	46·15	255·5	5·06
VO3	32	1·58	49·45	251·0	5·02
VO4	38	1·60	43·6	263·0	4·80
VO5	36	1·56	45·2	272·7	6·12
VO6	34	1·70	57·0	251·7	5·75
VO7	35	1·60	59·65	284·0	5·10
VO8	37	1·62	52·0	281·5	5·12
Mean	36·0	1·61	50·49	264·1	5·15
SD	2·3	0·005	5·29	12·7	0.54
University					
HO1	22	1·70	65·75	277·0	7·00
HO2	21	1·75	74·35	283·7	6·47
HO3	20	1·77	70·65	283·7	5·55
HO4	19	1·65	59·05	264·7	8·12
HO5	20	1·72	58·50	265·0	8·00
HO6	21	1·68	63·75	283·7	6·35
HO7	22	1·70	65·15	290·6	4·50
Mean	20·71	1·71	64·43	278·2	6·57
SD	1·11	0·004	5·46	9·2	1·20

Table 2. *Average composition of the low-protein diet**

Ingredients	Amount provided (g/d)	
	Village group	University group
Cassava (*Manihot esculenta*) (grated)	200	200
Maize starch	140	—
Custard flour	—	120
Refined cane sugar	30	50
Pepper (dried)	2	2
Tomato (fresh)	20	20
Onion (fresh)	20	20
Red palm oil	40	40
Okra (ladies fingers)	28	28
Common salt (iodized)	10	10
Orange drink	360	360
Caloreen powder (glucose polymer)	100	400
Chemical analysis		
Energy (MJ)	9·64	12·16
Protein (g) (nitrogen × 6·25)	4·68	4·68

* The composition of the diet as presented was calculated on the basis of the mean weight of each group.

The faecal samples were collected in labelled circular plastic containers with tightly fitting lids. The fresh samples were weighed before they were frozen for later analysis.

Integumental N losses

The medical students continued with their usual daily activities within the hospital wards and thus for aesthetic and procedural reasons, their integumental N losses could not be measured; however, N losses from the skin were determined in village adults. Subjects were first carefully washed with distilled water and then dressed in clean cotton clothes. After a 2 d interval, the subjects were washed again with clean distilled water and the clothes soaked with 0·2 M-sulphuric acid and N extracted. Portions of the bath-water were also analysed for N content and corrected for blank values. No attempt was made to determine N losses from the hair and nails.

Analytical methods

N contents of the diets, urine, faeces, bath-water and clothes were determined by the micro-Kjeldahl method as modified by Munro & Fleck (1969).

Data analysis

Endogenous N losses were calculated from the analysed N intake, daily urine total N for the last 5 d of the 10 d period and daily faecal N (estimated from the 5 d faecal pool). In addition, miscellaneous N losses were estimated from the summation of N lost in the bath-water and clothes. Calculation of protein requirements by the factorial method involved the summation of urinary, faecal and miscellaneous N losses along with the appropriate correction factors as suggested by the FAO/WHO (1973) report. Student's *t* test was used for comparing N results from the village and university subjects.

RESULTS

Table 3 summarizes daily urinary N losses from both the village adults and university students. All subjects demonstrated a consistent decrease in urinary N which stabilized after day 5 of the study. Table 4 shows the individual values for integumental N losses in the village subjects. A mean value of 7·46 mg N/kg per d was obtained for the group's integumental N losses. This value is much higher than the 5 mg N/kg per d used in one calculation of the FAO/WHO (1973) recommended protein allowances.

Table 5 provides values for the endogenous N losses and the apparent N balance of both the vilage adults and university students, both measured during the last 5 d of the 10 d period. There was no significant difference in urinary N expressed per kg body-weight between the two groups although the urinary N recorded in the present study is much higher than the 34 mg N/kg per d reported by Nicol & Phillips (1976*a*) in an earlier study in Nigeria. Again, there was no significant difference ($P > 0{\cdot}05$) in faecal N excretion between the two groups and the values obtained compared favourably with a faecal N of 23 mg N/kg per d obtained by Nicol & Phillips, (1976*a*). All subjects were in negative N balance. Table 6 summarizes the results of a factorial calculation of the protein allowance for both the village adults and university students and compares these results with those obtained for young adults (FAO/WHO, 1973) and adult Nigerian men (Nicol & Phillips, 1976*a*). Using the same correlation factors to adjust for efficiency of N utilization and to cover individual variability, the calculated protein allowance from the present study is much higher than that suggested by the FAO/WHO (1973) report for healthy young adults per kg body-weight.

Table 3. *Daily urinary nitrogen excretion (g/d) in Nigerian male adults fed on a low-protein diet*

	Period on diet (d)									
Subjects	1	2	3	4	5	6	7	8	9	10
Village										
VO1	2·83	2·64	2·54	2·62	2·46	2·63	2·37	2·88	2·44	2·08
VO2	3·63	2·57	2·52	2·59	3·78	2·82	2·27	2·38	1·95	2·67
VO3	3·85	3·60	2·85	2·48	2·76	2·32	2·23	2·34	1·97	2·20
VO4	3·05	2·75	2·21	2·04	2·18	1·98	2·01	2·06	2·10	2·52
VO5	3·60	2·56	1·96	2·28	2·38	2·06	2·14	2·46	2·60	2·07
VO6	2·62	2·66	3·25	3·05	2·66	2·24	2·60	2·04	2·10	2·47
VO7	7·06	2·74	2·84	2·79	2·58	2·60	2·71	2·26	2·05	1·89
VO8	—	2·93	2·53	1·92	2·45	2·35	1·92	2·24	2·53	2·38
Mean	3·81	2·81	2·59	2·47	2·66	2·37	2·28	2·33	2·22	2·21
SD	1·39	0·32	0·37	0·35	0·46	0·27	0·25	0·25	0·25	0·21
University										
HO1	7·86	5·76	5·85	4·76	3·32	2·85	3·345	2·88	2·44	3·90
HO2	7·19	5·96	4·30	3·92	2·97	2·80	2·81	3·68	3·83	3·90
HO3	5·54	5·78	3·87	3·48	3·28	3·30	2·80	2·65	2·78	2·87
HO4	5·73	3·94	2·64	2·41	2·71	2·98	2·78	2·38	2·70	2·64
HO5	5·58	4·94	3·92	2·52	2·73	2·73	2·52	2·87	2·03	2·18
HO6	—	3·68	4·14	3·36	2·70	2·51	2·96	2·52	2·90	—
HO7	—	4·68	4·09	2·88	2·31	2·53	2·80	2·58	2·73	—
Mean	6·38	4·96	4·12	3·33	2·86	2·81	2·86	2·79	2·77	3·10
SD	0·96	0·85	0·87	0·77	0·33	0·25	0·23	0·39	0·51	0·69

Table 4. *Integumental N losses in village subjects fed on a low-protein diet*

	Sources of N losses		Total N losses	
Subjects	Bath-water (mg)	Clothes (mg)	mg/d	mg/kg body-wt
VO1	11·25	499·80	511	10·04
VO2	12·91	396·08	408	8·84
VO3	12·91	436·10	449	9·08
VO4	4·59	453·45	258	5·93
VO5	4·03	225·90	230	5·09
VO6	4·53	413·50	418	7·33
VO7	7·92	372·10	380	6·37
VO8	7·92	355·10	363	6·98
Mean	8·25	369·00	377·13	7·46
SD	3·74	91·31	93·93	1·71

DISCUSSION

After correction for body-weight, the values for endogenous urinary and faecal N excretion did not vary significantly between the two groups of subjects in spite of the difference in age and socio-economic background, and they are in good agreement with the values reported for Nigerian men (Nicol & Phillips, 1976*a*) and Indian men (Gopalan & Narasinga Rao, 1966). The endogenous urinary N and faecal N excretion obtained in the present study were 34% and 26% higher for village adults and university students respectively when compared

Table 5. *Endogenous nitrogen excretion (mg N/kg per d) in Nigerian male adults fed on a low-protein diet*

Subjects	N intake	Urinary N	Faecal N	Urinary and faecal N	Apparent N balance
Village					
VO1	15·40	48·72	24·93	73·65	−58·25
VO2	16·99	51·79	23·99	75·78	−58·79
VO3	15·85	44·69	19·41	64·10	−48·25
VO4	17·98	49·08	25·00	74·08	−56·10
VO5	17·35	50·22	24·11	74·33	−56·99
VO6	13·75	40·17	20·52	60·69	−46·95
VO7	13·14	38·56	12·74	51·30	−38·16
VO8	15·08	43·84	23·65	67·49	−52·42
Mean	15·69	45·88	21·79	67·68	−51·99
SD	1·71	4·84	4·19	8·59	7·15
University					
HO1	13·62	46·84	24·86	71·70	−58·03
HO2	12·05	45·46	22·40	67·86	−55·82
HO3	12·68	40·76	15·07	55·83	−51·44
HO4	15·17	45·55	19·74	65·29	−50·13
HO5	15·20	41·88	21·00	62·88	−47·59
HO6	14·05	42·70	14·47	57·17	−43·09
HO7	13·75	40·98	10·69	51·67	−37·72
Mean	13·81	43·45	18·32	61·77	−49·12
SD	1·11	2·28	4·68	7·16	7·06

Table 6. *Factorial determination of protein requirements for various adult groups*

Group	Total mean obligatory* (mg/kg per d)	Adjusted N-requirements† (mg/kg per d)	Safe level of intake‡ N (mg/kg per d)	Safe level of intake‡ Protein (g/kg per d)
Young men§	54	70	91	0·57
Nigerian men‖	62	80	104	0·65
Village adults¶	75	97	126	0·78
University students¶	69	90	117	0·73

* Total obligatory N loss − urinary N + faecal N + miscellaneous N losses.
† Obligatory N losses are increased by 30% to account for efficiency of N utilization.
‡ Values are adjusted requirement plus 30% to allow for individual variability.
§ From Joint FAO/WHO *ad hoc* Expert Committee (1973).
‖ From Nicol & Phillips (1976*a*), assumes miscellaneous N losses to be 5 mg N/kg body-weight per d according to Joint FAO/WHO *ad hoc* Expert Committee (1973) recommendation.
¶ From the present study using a value of 7·46 mg N/kg body-weight per d as miscellaneous N losses determined for the village adults (this value was assumed for the university students).

with the value recorded in the FAO/WHO (1973) report for male adults. It is pertinent to note that the Indian and Nigerian diets are composed of low-protein vegetable foods of local origin which were domestically processed and may have contained high levels of crude fibre. On the other hand, the FAO/WHO (1973) report is based on studies in which the diets consisted mainly of purified and industrially processed foodstuffs. Thus, the significant difference in the endogenous urinary N and faecal N excretion in the Nigerian

male adults compared with the Caucasian adults may be due to the composition of the diets. Furthermore, the total obligatory losses obtained in the present study were at least 30% higher than the value recorded by the FAO/WHO (1973) (Table 5). Again, we obtained a slightly higher value of 7·46 mg N/kg per d for skin N losses as opposed to a value of 5·0 mg N/kg per d assumed by the FAO/WHO (1973). The present study was carried out during the cool period of the year (July–August) when room temperature was 24–28 ° and the relative humidity was 70–90%.

In the present paper, we have applied the factorial method to estimate the protein allowance for Nigerian male adults and to compare the values for this group with similar values from the FAO/WHO (1973) report. Calculation of protein requirements by the factorial method gives a value which is at least 30% higher than that reported by the FAO/WHO (1973). The endogenous urinary, faecal and skin N losses were higher in Nigerian subjects given a mixed vegetable, low-protein diet. Nicol & Phillips (1976*a*) suggested that it may not be possible to establish a single 'safe level of protein intake' based on body-weight and assuming an adequate energy intake, which would apply to all men of different ethnic, socio-economic and nutritional backgrounds. Indeed, man's requirements for protein are determined, to a considerable extent, by his ecological and socio-economic backgrounds and the proximate composition of the diets he customarily consumes.

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