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Nitrogen balance study in young Nigerian adult males using four levels of protein intake

BY T. ATINMO[1], C. M. F. MBOFUNG[1]†‡, G. EGUN[1]*
AND B. OSOTIMEHIN[2]

[1] *Department of Human Nutrition and* [2] *Department of Pathology, College of Medicine, University of Ibadan, Ibadan, Nigeria*

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1. The present study was carried out to estimate precisely, via the nitrogen balance technique, the protein requirement of Nigerians (earlier estimated via the obligatory N method) using graded levels of protein intake.

2. Fifteen medical students of the University of Ibadan who volunteered to participate in the study were given graded levels of protein (0·3, 0·45, 0·6 and 0·75 g/kg body-weight per d) derived from foods similar to those usually consumed by the subjects.

3. Each subject was given each of the dietary protein levels for a period of 10 d. Subjects were divided into two groups and the feeding pattern followed a criss-cross design with one group starting with the highest level of protein intake (0·75 g) and the second group starting with the lowest level of protein intake (0·3 g). Mean energy intake during each of the eleven experimental periods was maintained at 0·2 MJ/kg per d. After an initial 5 d adaptation period in each experimental period, 24 h urine and faecal samples were collected in marked containers for five consecutive days for N determination.

4. Mean N balance during consumption of the four protein levels (0·30, 0·45, 0·6 and 0·75 g/kg) were −11·02 (SD 8·07), −9·90 (SD 6·64), +9·70 (SD 4·15) and +5·13 (SD 4·62) respectively. Using regression analysis, the mean daily N requirement was estimated at 110·25 mg N/kg body-weight (0·69 g protein/kg body-weight). Estimates of allowances for individual variations to cover 97·5% of the population adjusted this value to 0·75 g protein/kg body-weight. Net protein utilization for the diet at maintenance level was estimated at 57·5.

The available internationally accepted information on protein requirements is based on premises not related to the physical biological and social factors unique to the Nigerian environment. The frequently cited minimum physiological nutrient requirement (the amount that is consistent with optimal health and above which no further improvement in health occurs) is at best a statistical approximation and usually derived from studies of privileged healthy Caucasians living under protected conditions.

The often-quoted studies of Nicol & Phillips (1976) in Nigeria suggested that there may be adaptive changes in protein requirements in chronically undernourished Nigerians. These studies, conducted in the late 1950s, showed that Nigerian males were in positive nitrogen balance during short-term periods on a rice-protein intake of 0·44 g/kg body-weight per d. Ever since the study by Nicol & Phillips (1976) relatively few studies have been made in Nigeria in this interesting area of nutrient requirement, which for several reasons requires further examination. In a recent study by Atinmo *et al.* (1985), protein requirements of healthy Nigerian males were estimated, via the obligatory N loss method, to be 0·75 g/kg per d. Compared with the recommended value of 0·57 g/kg per d proposed by the Joint FAO/WHO *ad hoc* Expert Committee on Energy and Protein Requirements (World Health Organization, 1973) this level of requirement is quite high.

The obligatory N loss method of estimating protein requirement is, however, defective

Present addresses: * Department of Biochemistry, Lagos State University, Lagos, Nigeria.
† Department of Food Science and Nutrition, ENSIAAC, Ngaoundere University Centre, Ngaoundere, Cameroon.
‡ For reprints.

in that it assumes many factors. It has been demonstrated in healthy adults that N retention cannot be obtained by providing high quality protein at an N level that is equal to the summated total obligatory N losses (Calloway & Merger, 1971; Young *et al.* 1973). Additional N is usually required. Thus the N balance technique of giving graded levels of protein has been suggested to be the most direct way of estimating protein needs. It involves the assessment of N balance responses to graded levels of protein intake, within the limits of submaintenance to maintenance N intake for adults or children over a range of intake levels that is close to those required for maintenance and normal growth (Garza, *et al.* 1977; Kishi *et al.* 1978; Oddoye & Margen, 1979). This method, in principle, takes care of the effect of non-physiological conditions in the factorial method.

Considering the advantage of the N balance method, and above all the fact that protein requirements differ among similar individuals, just as food protein sources vary in their capacity to meet these protein requirements (Garza *et al.* 1977, Kishi *et al.* 1978), it was deemed necessary to define more precisely the protein requirements of Nigerians consuming ordinary local mixed foods under customary conditions of daily life. This was the main objective of the present study.

MATERIALS AND METHOD

Subjects

Fifteen healthy medical students of the University of Ibadan were recruited into the study. They were aged 19–21 years and weighted between 54 and 69 kg (average 60·75 kg). Their health status was evaluated on the basis of medical history, physical examination and routine laboratory tests. The subjects were fully informed of the aim, nature and design of the experiment and they signed consent forms. They were studied under free-living conditions with close medical supervision. Daily body-weights were recorded at 07.00 hours throughout the entire study under standardized conditions (preprandial, post-voiding and with light indoor clothing). The subjects were engaged in their normal routine daily activities while maintaining a reasonable constant level of physical activity during the study period. The physical activities of the subjects were closely monitored by regularly checking a diary of daily activities which was kept by each subject.

Diet and experimental design

Diets were standardized, based on foods usually consumed by subjects (Table 1), and given in a meal pattern, three times daily at 07.00–08.00, 13.00–14.00 and 19.00–20.00 hours. Complete vitamin and mineral supplements were added to ensure that no vitamins or minerals were limiting. The study lasted 54 d and consisted of four experimental diet periods, each of 10 d duration in which four different dietary protein levels were given (0·30, 0·45, 0·60 and 0·75 g protein/kg per d).

All the subjects were tested on the different levels of protein intake. To achieve this end, some of the subjects (subjects OS, AS, FA, KU, MA, OP, DU and HA) were assigned to an ascending sequence of dietary protein intake while the rest were assigned to a descending sequence. Their energy intake was carefully determined by the dietician based on a careful history of dietary habits, body-weight and evaluation of the physical activity of each of the subjects. This was fixed at a level of 0·2 MJ/kg body-weight per d throughout the experiment.

A protein-free diet was given on the 1st day before the beginning of each dietary period to promote rapid adaptation to the experimental diet. A 3 d break period with a free-choice diet separated each of the four experimental diet periods. During the experimental diet periods, the subjects were required to adhere to the experimental diet and thus eat only the food prepared by the dietician.

Table 1. *Composition of diet*

	Amount provided (g/d)
Cassava (*Manihot esculenta*) grated	130
Beef	116
Vegetables	14
Rice (cooked)	400
Bread	120
Refined sugar	30
Magarine	30
Tomatoes (fresh)	80
Palm oil	40
Onions	40
Peppers (dried)	4
Salt	Added to taste
Orange drink	Three bottles
Nutrient content	
Protein (g)	45·22
Energy (MJ)	11·87

Sampling and analysis

Complete 24 h urine samples were collected daily thoughout the entire experimental period using hydrochloric acid as the preservative. Completenes of the urine collection was evaluated by analysing the urine for creatinine.

During each of the last 5 d of the 10 d period of each experimental diet, complete faecal samples were collected in individual containers, stored frozen and later combined and homogenized as a 5 d pooled sample for each subject. A carmine dye capsule was used as the faecal marker in each period. Food samples were collected in plastic bags, weighed, homogenized and portions taken and frozen.

N determination in food, urine and faecal samples was by a micro-Kjedahl method as modified by Munro & Fleck (1969). Values obtained were evaluated statistically, including regression analysis and analysis of variance (ANOVA).

For the last 5 d of each 10 d experimental diet period, N balance was calculated from intake, and faecal, urine and miscellaneous losses (skin N loss was taken as 7·46 mg N/kg body-weight per d from a previous determination (Atinmo *et al.* 1985)).

Estimates of individual N requirements was by linear regression equation relating N intake to balance (Rand *et al.* 1979). The protein intake at which N balance was at equilibrium was estimated as the N requirement. The biological value (BV) and the net protein utilization (NPU) of the diets were calculated by conventional methods. Obligatory urinary and faecal N losses were taken as 43·42 and 18·32 mg N/kg respectively after Atinmo *et al.* (1985).

RESULTS

Table 2 shows the N excretion and balance of individual subjects. All subjects demonstrated increase in urinary N levels with increases in N intake. These changes were statistically significant ($P < 0.05$; Table 3). N balance became more positive with increasing N intake. The changes in individual apparent N balance for each of the subjects is as shown in Table 2 while the estimated true balance is summarized in Table 3. As a group, subjects showed negative N balance when on a daily protein intake level of 0·30 and 0·45 g/kg body-weight per d. The degree of negative balance at each of these two levels of protein intake was significantly different when the order in which the protein was given was considered (Table

Table 2. *Nitrogen excretion and balance (mg/kg body-weight per d) in Nigerian college students*

N intake...	55·55			77·42			100·36			130·34		
Subject	UN	FN	B	UN	FN	B	UN	FN	B	UN	FN	B
OS	48·83	25·32	−18·19	70·13	25·12	−15·38	—	—	—	106·14	26·72	+2·26
AS	71·16	10·73	−27·55	71·81	23·36	−17·19	78·39	31·34	+1·95	103·42	25·97	+1·17
FA	45.04	21.61	−16.2	63.90	18.58	−10.60	75.68	22.96	+1.02	—	—	—
KU	53.51	16.80	−14.44	74.08	23.68	−19.07	74.58	27.12	+6.02	99.66	26.97	+5.3
MA	45.51	29.11	−17.53	73.37	27.07	−19.97	81.20	25.36	+3.69	111.39	22.65	+0.66
OP	55.67	16.03	−15.51	63.77	30.09	−13.47	83.20	26.05	+0.54	107.64	26.97	−0.30
DU	48.47	17.46	−15.64	54.83	29.38	−12.34	64.76	25.97	+7.52	90.28	18.80	+10.34
HA	48.39	19.28	−12.86	66.40	23.03	−10.99	80.22	27.65	+0.89	104.03	27.99	+1.78
IW	35.58	25.32	−3.89	62.32	19.80	−2.97	66.80	23.39	+6.76	96.46	25.92	+7.81
EG	54.23	17.82	−16.96	59.49	18.57	−3.23	69.87	26.78	−4.50	91.49	22.49	+7.24
OM	43.83	17.76	−4.59	60.83	20.30	−2.60	69.79	32.58	−5.90	90.84	24.48	+15.28
OB	40.31	14.87	−2.11	59.82	18.46	−4.01	64.99	19.22	+7.3	89.20	26.02	+8.99
OR	43.11	15.27	−4.83	57.28	19.56	−4.22	67.83	26.02	−3.49	—	—	—
UD	47.85	16.27	−3.44	63.50	25.88	−8.21	78.16	23.60	−1.94	106.57	25.15	+1.96
EK	49.06	16.29	−5.11	61.80	24.82	−4.12	73.62	26.00	+3.35	98.51	26.61	+10.36
Mean	48·14	18·38	−11·02	65·32	23·38	−9·90	72·75	25·89	+1·97	100·09	25·38	+5·13
SD	8·18	4·75	8·07	6·32	3·86	6·64	6·15	3·18	4·15	7·15	2·72	4·62

UN, urinary N; FN, faecal N; B, N balance.

Table 3. *Daily nitrogen values (mg/kg body-weight per d) with increasing N intake in Nigerian college students*

(Mean values and standard deviations for fifteen subjects)

NI		UN		FN		APP.NB		EST.TNB	
Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
55·55	2·80	48·1	8·18	18·38	4·75	−11·02	8·07	−18·48	8·07
77·42	3·29	65·32	6·32	23·38	3·86	−9·90	6·64	−17·36	6·67
100·36	6·76	72·75	6·15	25·89	3·18	+1·97	4·15	−5·49	4·15
130·34	4·96	100·09	7·15	25·38	2·72	+5·13	4·62	−2·33	4·62

NI, N intake; UN, urinary N; FN, faecal N; APP.NB, apparent N balance (NI−UN−FN); EST.TNB, estimated true N balance assuming N losses from sweat as 7·46 mg N/kg body-weight per d from previous estimates by Calloway & Merger (1971).

Within vertical columns, mean values were significantly different from the other values (one-way ANOVA): $P < 0{\cdot}05$

4). On a protein intake of 0·60 g/kg body-weight per d, ten of the subjects achieved an apparent positive N balance and, when placed on a daily protein intake of 0·75 g/kg body-weight, all subjects except one were in positive N retention. The range of the N balance (excluding estimated losses through sweat) was −0·30–15·8 mg N/kg body-weight at the 0·75 g/kg body-weight level of protein intake.

Estimated true balance (that includes estimated skin N loss of 7·46 mg N/kg body weight per d) resulted in a mean negative N balance of −2·3 mg N/kg per d even at the high-protein intake level of 0·75 g protein/kg body-weight.

Table 4. *Nitrogen balance (mg/kg body-weight per d) according to order of protein intake*

(Mean values and standard deviations)

		N balance (mg/kg body-wt per d)							
N intake (mg/kg body-wt per d)...		55·5		77·42		100·36		130·34	
Order*	Subjects	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Ascending	OS–HA	−17·3	4·5	−14·9	3·6	3·1	2·8	1·8	1·9
Descending	IW–EK	− 5·9	5·0	− 4·2	1·9	−1·9	5·0	8·6	4·3
Statistical significance (*t* test): $P <$	—	0·001		0·001		NS		0·05	

NS, Not significant.

* Order of intake of dietary protein, from 55·55 to 130·34 or from 130·34 to 55·55 mg N/kg body-weight per d.

Table 5. *Linear regresssion equations relating nitrogen balance* (Y) *to N intake* (X) *for each of the Nigerian college students studied*

Subject	Regression equation	Predicted mean intake to achieve N balance
OS	$Y = 0{\cdot}27X - 34{\cdot}85$	129.11
AS	$Y = 0{\cdot}42X - 49{\cdot}34$	117·47
FA	$Y = 0{\cdot}35X - 34{\cdot}41$	98·32
KU	$Y = 0{\cdot}34X - 37{\cdot}41$	110·02
MA	$Y = 0{\cdot}31X - 38{\cdot}03$	122·69
OP	$Y = 0{\cdot}23X - 29{\cdot}14$	126·4
DU	$Y = 0{\cdot}43X - 39{\cdot}1$	90·93
HA	$Y = 0{\cdot}21X - 25{\cdot}0$	118·96
IW	$Y = 0{\cdot}18X - 14{\cdot}39$	79·93
EG	$Y = 0{\cdot}33X - 32{\cdot}7$	98·94
OM	$Y = 0{\cdot}26X - 22{\cdot}93$	88·20
OB	$Y = 0{\cdot}19X - 13{\cdot}68$	72·02
OR	$Y = 0{\cdot}04X - 7.49$	187.18
UD	$Y = 0{\cdot}10X - 12{\cdot}32$	123·29
EK	$Y = 0{\cdot}22X - 19{\cdot}84$	90·20
Mean	$Y = 0{\cdot}22X - 19{\cdot}82$	110·25 (SD 27.9)
	Pooled regression	$Y = 0{\cdot}186X - 20{\cdot}12$; if $Y = 0$, X = 108·18 mg/kg

Results of individual regression analysis of N balance (apparent) on N intake are as shown in Table 5. Mean intake predicted to achieve N-balance was 108·18 mg N/kg body-weight per d with a range of 72·02–187·18 mg N/kg body-weight. The mean protein requirement, including the estimated sweat and skin losses of 7·46 mg N/kg body-weight, was estimated as 117·71 mg N/kg body-weight per d.

Calculations of the maintenance protein requirement were performed with the N balance values using the principle of the multiple-level individual responses method of Rand *et al.* (1979).

The mean protein requirement of the subjects was thus 110·25 mg N/kg body-weight per d or 0·60 g protein/kg per d. Estimates for individual variations to cover 97·5 % of the population adjusted this value to 0·75 g protein/kg body-weight per d.

Table 6. *Biological value (BV), net protein utilization (NPU) and true and apparent digestibilities of the diet at different levels of protein intake for Nigerian college students*

(Mean values and standard deviations)

Level of protein intake (g/kg body-wt per d)	BV		NPU		Digestibility			
					Apparent		True	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0·30	91·77	11·75	90·79	11·04	66·28	8·57	99·42	8·56
0·45	71·47	7·91	67·19	9·16	70·17	4·81	93·87	5·09
0·60	68·24	4·88	63·10	5·58	74·19	3·31	92·2	3·30
0·75	54·63*	4·92	51·82*	5·14	80·75NS	1·53	94·82NS	1·86

NS, not significant, $P > 0·05$.
* Mean values were significantly different from the other values in the column (one-way ANOVA): $P < 0·05$.

The BV, NPU and the true and apparent digestibilities for the dietary protein at the different levels of N intakes are summarized in Table 6. The BV and NPU were significantly reduced ($P < 0·05$) with increase in protein intake. Digestibilities (true and apparent) were not significantly affected by increasing protein intake. However, values of apparent digestibility were relatively lower at low-protein intake levels than at higher levels.

DISCUSSION

The N balance method was used in the present study to estimate the protein requirements of Nigerian adult males. Apart from the earlier studies made by Nicol & Phillips (1976), the obligatory N loss method was used recently to estimate the protein requirement of Nigerians (Atinmo *et al.* 1985). The subjects who participated in the present study were young, healthy, medical students and thus provided a basis for comparison of our results with those of other studies elsewhere in the world. In particular, since none of the subjects had any disease condition that could contribute to an increased protein requirement during the whole period of the experiment, our results can be compared with those of Caucasians.

N balance was observed with increasing protein intake level from the submaintenance to the maintenance level. Although the criss-cross design adopted here for the feeding of the different protein levels has its advantages, it is pertinent to note that the order in which the subjects received the protein did influence their response in terms of N balance; moving from a higher to a lower protein level seemed to have a different effect on N balance than moving from a lower to a higher level of protein intake. The individual responses to the different levels of N intake could not strictly be defined by a straight line according to the Rand's model (Rand *et al.* 1979). Studies in experimental animals and man have also shown that N balance response is not linear throughout the entire submaintenance range (Inoue *et al.* 1973; Young *et al.* 1973). Nonetheless, the variation in the individual responses, observed in the present study, seem to underscore the fact that at lower protein intake level, adaptive mechanisms tend to occur and that such adaptive mechanisms may also be influenced by the order in which the protein levels are given.

The subjects showed a cumulative apparent positive N balance from intakes of about 0·60–0·70 g protein/kg body-weight. The sum of obligatory urinary and metabolic faecal N losses with an appropriate allowance for sweat and integumental losses obtained in the recent study by Atinmo *et al.* (1985) was 69·23 mg N/kg body-weight. This value is much lower than that of 110·25 mg N/kg body-weight per d obtained in the present study through regression analysis of the values for individual subjects on N balance. Furthermore, it is necessary to point out that the present study did not include the estimation of miscellaneous N losses, thus the real maintenance requirement, taking into consideration an average skin N loss of 7·46 mg N/kg obtained from the study by Atinmo *et al.* (1985), would be 117·71 mg N/kg per d. This difference in estimates emphasizes the importance of using total losses of N in estimating requirements levels. As pointed out by Wallace (1959), error inherent in the N balance method could lead to an underestimation of total N losses and thus to an overestimation of N retention and underestimation of protein requirements. Based on our results and those of Atinmo *et al.* (1985), it seems that the obligatory N loss method tends to underestimate the minimum requirement for protein, if adaptive metabolic changes occur throughout the submaintenance range of protein intakes.

The present estimate of 0.69 g protein/kg per d is higher than values obtained using single protein sources for Caucasians and orientals (Young *et al.* 1973; Huang & Lin, 1982) but compares favourably with results from orientals receiving a mixed-vegetable diet (Huang & Lin, 1982).

The safe level of protein or N intake includes an allowance for covering individual variation. This is the mean requirement plus two standard deviations which is believed to satisfy the needs of nearly all (97·5 %) the population. From our results, such a safe level of intake was estimated as 0·75 g protein/kg body-weight. This value, even when adjusted for the quality of the protein consumed, is much higher than that of 0·57 g protein/kg body-weight recommended by the Joint FAO/WHO *ad hoc* Expert Committee on Protein Requirements, (World Health Organization, 1973) and corresponds to the value given by the most recent report of the Food and Agriculture Organization/World Health Organization/United Nations University, 1985).

Utilization of protein-N in the diets was high at 0·38 g protein/kg body-weight but decreased significantly with increasing protein intake, as suggested by the BV and NPU estimates. This inferred a decreased efficiency in protein utilization as the maintenance level of protein intake was approached. These results fully support the findings of Young *et al.* (1973) and those of Inoue *et al.* (1973). Utilization of protein consumed at a level just adequate to meet requirements is, however, significantly less efficient than would be assumed from biological determinations of protein value in animals and man. This is borne out by the need to supply more protein to achieve N balance than factorial measurement of total obligatory N loss would predict (Young *et al.* 1973). Based on the present study, the NPU of Nigerian local diets at near maintenance levels may be estimated as the mean value of NPU at the protein intake levels of 0·6 and 0·75 g/kg, that is 57·5 g/kg. This value compares favourably with that of egg protein, as given by Kishi *et al.* (1978) and Huang & Lin (1982). From these findings the efficiency of dietary protein utilization at the maintenance level may be considered to be about 60 % in young Nigerian men.

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