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

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

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

3. Each subject was given each of the dietary protein levels for a period of 10 d. Subjects were divic groups and the feeding pattern followed a criss-cross design with one group starting with the highest lev intake (0·75 g) and the second group starting with the lowest level of protein intake (0·3 g). M intake during each of the eleven experimental periods was maintained at 0·2 MJ/kg per d. After a adaptation period in each experimental period, 24 h urine and faecal samples were collected in marke 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) v (SD 8·07), −9·90 (SD 6·64), +9·70 (SD 4·15) and +5·13 (SD 4·62) respectively. Using regression analys daily N requirement was estimated at 110·25 mg N/kg body-weight (0·69 g protein/kg body-weight). allowances for individual variations to cover 97·5% of the population adjusted this value to 0·75 g 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 premises not related to the physical biological and social factors unique to the environment. The frequently cited minimum physiological nutrient requirer amount that is consistent with optimal health and above which no further impro health occurs) is at best a statistical approximation and usually derived from privileged healthy Caucasians living under protected conditions.

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

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

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

Considering the advantage of the N balance method, and above all the fac
requirements differ among similar individuals, just as food protein sources
capacity to meet these protein requirements (Garza *et al.* 1977, Kishi *et al.*
deemed necessary to define more precisely the protein requirements of Nigeria
ordinary local mixed foods under customary conditions of daily life. This
objective of the present study.

MATERIALS AND METHOD

Subjects

Fifteen healthy medical students of the University of Ibadan were recruited i
They were aged 19–21 years and weighted between 54 and 69 kg (average 60
health status was evaluated on the basis of medical history, physical exa
routine laboratory tests. The subjects were fully informed of the aim, nature
the experiment and they signed consent forms. They were studied unc
conditions with close medical supervision. Daily body-weights were reco
hours throughout the entire study under standardized conditions (prep
voiding and with light indoor clothing). The subjects were engaged in their n
daily activities while maintaining a reasonable constant level of physical acti
study period. The physical activities of the subjects were closely monitore
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 (Tabl
in a meal pattern, three times daily at 07.00–08.00, 13.00–14.00 and 19.00
Complete vitamin and mineral supplements were added to ensure that n
minerals were limiting. The study lasted 54 d and consisted of four exp
periods, each of 10 d duration in which four different dietary protein levels we
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 ac
some of the subjects (subjects OS, AS, FA, KU, MA, OP, DU and HA) we
an ascending sequence of dietary protein intake while the rest were assigned t
sequence. Their energy intake was carefully determined by the dietician base
history of dietary habits, body-weight and evaluation of the physical activity
subjects. This was fixed at a level of 0·2 MJ/kg body-weight per d th
experiment.

A protein-free diet was given on the 1st day before the beginning of each
to promote rapid adaptation to the experimental diet. A 3 d break period wit
diet separated each of the four experimental diet periods. During the exp
periods, the subjects were required to adhere to the experimental diet and th
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 experimen using hydrochloric acid as the preservative. Completenes of the urine colle evaluated by analysing the urine for creatinine.

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

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

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

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

RESULTS

Table 2 shows the N excretion and balance of individual subjects. All subjects den increase in urinary N levels with increases in N intake. These changes were st significant ($P < 0{\cdot}05$; Table 3). N balance became more positive with increasing The changes in individual apparent N balance for each of the subjects is as show 2 while the estimated true balance is summarized in Table 3. As a group, subjec negative N balance when on a daily protein intake level of 0·30 and 0·45 g/kg bo per d. The degree of negative balance at each of these two levels of protein i significantly different when the order in which the protein was given was conside

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

N intake...	55·55			77·42			100·36			
Subject	UN	FN	B	UN	FN	B	UN	FN	B	UN
OS	48·83	25·32	−18·19	70·13	25·12	−15·38	—	—	—	106·14
AS	71·16	10·73	−27·55	71·81	23·36	−17·19	78·39	31·34	+1·95	103·42
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
MA	45.51	29.11	−17.53	73.37	27.07	−19.97	81.20	25.36	+3.69	111.39
OP	55.67	16.03	−15.51	63.77	30.09	−13.47	83.20	26.05	+0.54	107.64
DU	48.47	17.46	−15.64	54.83	29.38	−12.34	64.76	25.97	+7.52	90.28
HA	48.39	19.28	−12.86	66.40	23.03	−10.99	80.22	27.65	+0.89	104.03
IW	35.58	25.32	−3.89	62.32	19.80	−2.97	66.80	23.39	+6.76	96.46
EG	54.23	17.82	−16.96	59.49	18.57	−3.23	69.87	26.78	−4.50	91.49
OM	43.83	17.76	−4.59	60.83	20.30	−2.60	69.79	32.58	−5.90	90.84
OB	40.31	14.87	−2.11	59.82	18.46	−4.01	64.99	19.22	+7.3	89.20
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
EK	49.06	16.29	−5.11	61.80	24.82	−4.12	73.62	26.00	+3.35	98.51
Mean	48·14	18·38	−11·02	65·32	23·38	−9·90	72·75	25·89	+1·97	100·09
SD	8·18	4·75	8·07	6·32	3·86	6·64	6·15	3·18	4·15	7·15

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

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

(Mean values and standard deviations for fifteen subjects)

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

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

Within vertical columns, mean values were significantly different from the other values (on
$P < 0·05$

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

Estimated true balance (that includes estimated skin N loss of 7·46 mg N/k
per d) resulted in a mean negative N balance of −2·3 mg N/kg per d eve
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 prot*

(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	
Order*	Subjects	Mean	SD	Mean	SD	Mean	SD
Ascending	OS–HA	−17·3	4·5	−14·9	3·6	3·1	2·8
Descending	IW–EK	− 5·9	5·0	− 4·2	1·9	−1·9	5·0
Statistical significance (t test): $P <$	—	0·001		0·001		NS	

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 per d.

Table 5. *Linear regresssion equations relating nitrogen balance (Y) to N intak 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 int shown in Table 5. Mean intake predicted to achieve N-balance was 108·18 mg N weight per d with a range of 72·02–187·18 mg N/kg body-weight. The mea requirement, including the estimated sweat and skin losses of 7·46 mg N/kg bo was estimated as 117·71 mg N/kg body-weight per d.

Calculations of the maintenance protein requirement were performed with the values using the principle of the multiple-level individual responses method of F (1979).

The mean protein requirement of the subjects was thus 110·25 mg N/kg body- d or 0·60 g protein/kg per d. Estimates for individual variations to cover 97· 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 anc digestibilities of the diet at different levels of protein intake for Nigerian coll*

(Mean values and standard deviations)

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

NS, not significant, $P > 0·05$.

* Mean values were significantly different from the other values in the column (one-way AN(

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

DISCUSSION

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

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

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

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

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

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

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