Long-term evaluation of the adequacy of habitual diets to p protein needs of adult Nigerian men

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1. A continuous 40 d metabolic nitrogen balance study was conducted on twelve young men age years to evaluate the adequacy of a previously determined safe level of protein intake.

2. Subjects were fed on diets which they were habitually accustomed to and which provided a pr 0·75 g protein (N × 6·25)/kg body-weight per d as the safe level and an energy intake level of 0·2 M weight per d. N balances, including an estimate for integumental losses as well as certain biochemic were determined for the last 5 d of two consecutive 20 d diet periods.

3. Only two of the twelve subjects were observed to be in negative N balance during the final 5 period. N balance was generally positive at 8·24 (SD 8·61) mg N/kg body-weight, thus confirming t of 0·75 g protein/kg body-weight per d as a safe level of protein intake in the majority of the subj

Attempts have been made using different approaches to quantify the amount o needed by man for growth and maintenance of health. Two physiological appro used by the Joint FAO/WHO *ad hoc* Expert Committee on Energy an Requirements of man (World Health Organization, 1973): these are the factorial based on results from short-term, N-free diets fed to young adults, and the appro on the use of results from linear extrapolation of short-term N balance studies adults fed on graded levels of protein (Huang & Lin, 1982).

It is now becoming clear that short-term N balance determinations alone provide an adequate measure of dietary protein needs or of changes in nutritio Measurement of N balance is simply an overall summation of body N change. I no information on the occurrence and significance of either beneficial or c adaptations that may arise from possible alterations in the pattern of N distrit metabolism within the body (Yoshimura, 1972). From this point of view, therefo of N balance studies of longer duration seem more desirable and acceptable, as in derived from them would include the effect of any forms of adaptations not short-term studies, such as those recently used to determine the protein requi adult and young adult Nigerians (Atinmo *et al.* 1981, 1988). In the earlier study *et al.* (1981), the protein requirement of the subjects was estimated at 0·60 g weight per d, while the safe level of protein intake was suggested to be 0·75 weight per d.

This safe level of protein intake was also obtained from the extrapolation of th an N balance study in young Nigerians using graded levels of protein intake (At 1988). However, since this value in both cases was based on the results of studies, further testing under free-living conditions and for a longer period w necessary. The objective of the present study was to evaluate the adequacy of th of protein intake (0·75 g/kg body-weight per d) in adult Nigerian men consu habitual diets containing this level of protein for a period of 40 d.

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MATERIALS AND METHODS

Twelve young university men, between the ages of 22 and 29 years, partic study. All were free from any detectable medical or physical abnormalitie characteristics of the subjects are shown in Table 1. The subjects were studie supervision in their nearby hall of residence and were engaged in their norm daily activities, which included their full academic schedule while maintaining constant level of physical activity.

Daily body-weights were recorded at 07.00 hours throughout the entire under standardized conditions (preprandial, post-voiding, and with light indc

Diets

The composition of the diet, which was similar to the habitual diets of th described by Atinmo *et al.* (1988). The basic formula was the same for all subj to meet individual energy requirements without increasing the protein level o individual energy intake of 0·2 MJ/kg body-weight was met in each case by or removal from the basic diet formula, of an extra energy source in the form flavoured drink. The diet provided 0·75 g protein/kg body-weight per d whicl level of protein estimated from a short-term N balance study by Atinmo *et a* diets were served in a meal pattern that was familiar to the subjects, 07.00–08.00, 13.00–14.00 and 19.00–20.00 hours.

Throughout the study, all subjects were required to adhere strictly to the diets and not to eat anything outside the diets provided. For the purpose adaptations to the test protein level, the 40 d experimental period was divi consecutive 20 d diet periods for N balance studies.

The study plan was approved by the Ethical Committee of the College of subjects signed consent forms.

Sampling and measurement

Daily urinary and faecal samples were collected as voided according to strict later analysed as in a previous study (Atinmo *et al.* 1985). The collection of th done on days 16–20 and 36–40 of the study.

Fasting blood samples were drawn from the antecubital vein into hepa which were then centrifuged and analysed according to standard biochem using specific reagent kits for each type of analysis. Sweat N losses were co methods of Sirbu *et al.* (1967) and of Spence *et al.* (1972).

Anthropometric measurements to detect possible changes in body com made. These measurements included mid-arm muscle circumference thicknesses at triceps, subscapular and suprailiac sites.

N balance

N balance (mg N/kg body-weight per d) was calculated from the analysed N daily urinary N for the last 5 d of each 20 d period (UN) and total daily faecal from the 5 d faecal pool, FN). In addition a factor to allow for miscellan through sweat was also included in the calculations.

RESULTS

A comparison of the body-weight of the subjects before and during each of tl periods showed no significant difference (Table 2). Anthropometric measuren the beginning, in the middle and at the end of the study (Table 3) also did significant changes.

Table 1. *Characteristics of the adult Nigerian male subjects*

Subject	Age (years)	Wt (kg)	Height (m)	BMR (MJ/d)
M1	29	62·0	1·63	7·23
M2	27	57·0	1·63	6·89
M3	27	57·0	1·64	6·89
M4	25	56·0	1·68	6·96
M5	26	58·0	1·68	6·96
M6	23	61·0	1·71	7·17
M7	28	69·0	1·72	7·71
M8	22	64·5	1·71	7·40
M9	22	49·5	1·55	6·38
M10	22	60·0	1·76	7·10
M11	22	73·5	1·75	8·02
M12	26	61·0	1·76	7·17
Mean	25	60·7	1·68	7·16
SD	2·6	6·3	0·68	0·42

BMR, basal metabolic rate calculated by the World Health Organization (1973) method

Table 2. *Body-weights (kg) of twelve adult Nigerian men consuming their usual 40 d (periods 1 and 2, each of 20 d)*

Subject	Initial body-wt	Period 1*	Period 2*
M1	62·0	60·5	60·0
M2	57·0	54·0	54·0
M3	57·0	57·0	57·0
M4	56·0	56·0	55·0
M5	58·0	57·5	57·5
M6	61·0	60·0	60·0
M7	69·0	68·5	69·0
M8	64·5	61·0	62·0
M9	49·5	49·0	49·0
M10	60·0	58·5	59·0
M11	73·5	70·0	67·5
M12	61·0	60·5	60·5
Mean	60·7	59·4	59·2NS
SD	6·3	5·7	5·5

NS, no significant difference in body-weight ($P > 0·05$).
* Average body-weight of the last 5 d of each period.

N balance (Table 4) was calculated with integumentary N loss measured at 1 kg body-weight per d. N balance for the young men during the two succe experimental periods was 6·59 (SD 6·93) and 8·24 (SD 8·61) mg N/kg bo respectively. During the last 5 d of the initial 20 d period, only two subjects were i N balance. The difference in N balance between the two periods was not s Urinary creatinine (Table 5) measured during the last 5 d of each 20 d period w an index of the consistency and completeness of urine collections. Generally th change in the daily urinary creatinine excretion of the twelve subjects durin consecutive 20 d periods. Also the relative contribution of urinary N to total N which was about 80%, was unchanged throughout the study.

Table 3. *Anthropometric measurements of young adult Nigerian males at th middle and end of the long-term study*

(Mean values and standard deviations)

Stage of study...	Beginning		Middle		
	Mean	SD	Mean	SD	M
Wt (kg)	60·7	6·3	59·4	5·7	5
Mid-arm muscle circumference (mm)*	267·0	12·0	267·0	12·0	26
Waist circumference (mm)	720·0	29·0	714·0	34·0	71
Total skinfold thickness (mm)	36·6	10·5	35·9	10·8	3
Percentage body fat*	15·6	3·2	15·3	3·4	1

NS, not significantly different ($P > 0·05$) from the values at the beginning of the st
* Calculated after the method of Gurney & Jelliffe (1973).

Table 4. *Nitrogen balance (mg/kg per d) of twelve adult Nigerian males con usual diet for 40 d (periods 1 and 2, each of 20 d)*

Subject	NI	UN	FN	TN	N balance
		Period 1			
M1	119·1	84·0	15·8	110·13	8·95
M2	131·6	87·7	21·8	119·95	11·60
M3	128·1	88·2	13·3	111·87	16·18
M4	130·4	85·2	29·1	124·78	5·58
M5	125·8	81·7	31·0	123·07	2·38
M6	119·6	85·3	26·3	121·97	−2·38
M7	104·0	75·8	15·2	101·43	3·43
M8	116·7	78·1	25·7	114·27	2·42
M9	147·7	87·8	32·4	130·42	17·13
M10	122·6	74·5	30·0	114·93	7·70
M11	102·0	73·0	24·1	107·46	−5·56
M12	118·6	83·0	13·9	107·34	11·27
Mean	122·23	82·02	23·22	115·64	6·59
SD	12·14	5·40	7·06	8·55	6·93
		Period 2			
M1	120·58	84·69	20·36	115·45	5·13
M2	133·98	81·68	20·65	112·73	21·25
M3	125·83	85·07	15·03	116·11	9·72
M4	131·55	88·79	27·34	121·51	10·04
M5	124·74	82·62	30·40	123·42	1·32
M6	120·58	79·71	29·56	119·67	0·91
M7	104·86	74·80	14·36	99·56	5·30
M8	116·96	83·57	24·39	118·36	−1·67
M9	147·65	86·33	26·98	123·70	23·95
M10	122·63	75·80	30·58	106·77	15·86
M11	106·40	74·49	23·05	108·94	−2·54
M12	118·61	85·64	12·93	108·97	9·64
Mean	122·84	81·93	22·97	114·60	8·24
SD	11·63	4·76	6·35	7·42	8·61

NI, nitrogen intake; UN, urinary N; FN, faecal N; TN, total N including integumentary N at 10·4 mg N/kg per d.

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M4	56·0	56·0	55·0
M5	58·0	57·5	57·5
M6	61·0	60·0	60·0
M7	69·0	68·5	69·0
M8	64·5	61·0	62·0
M9	49·5	49·0	49·0
M10	60·0	58·5	59·0
M11	73·5	70·0	67·5
M12	61·0	60·5	60·5
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M8	116·7	78·1	25·7	114·27	2·42
M9	147·7	87·8	32·4	130·42	17·13
M10	122·6	74·5	30·0	114·93	7·70
M11	102·0	73·0	24·1	107·46	−5·56
M12	118·6	83·0	13·9	107·34	11·27
Mean	122·23	82·02	23·22	115·64	6·59
SD	12·14	5·40	7·06	8·55	6·93
		Period 2			
M1	120·58	84·69	20·36	115·45	5·13
M2	133·98	81·68	20·65	112·73	21·25
M3	125·83	85·07	15·03	116·11	9·72
M4	131·55	88·79	27·34	121·51	10·04
M5	124·74	82·62	30·40	123·42	1·32
M6	120·58	79·71	29·56	119·67	0·91
M7	104·86	74·80	14·36	99·56	5·30
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M9	147·65	86·33	26·98	123·70	23·95
M10	122·63	75·80	30·58	106·77	15·86
M11	106·40	74·49	23·05	108·94	−2·54
M12	118·61	85·64	12·93	108·97	9·64
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NI, nitrogen intake; UN, urinary N; FN, faecal N; TN, total N including integumentary N at 10·4 mg N/kg per d.

Table 7. *Protein quality indices of habitual diets consumed by twelve adult for 40 d (periods 1 and 2, each of 20 d)*

(Mean values and standard deviations)

Indices	Period 1		Period 2	
	Mean	SD	Mean	SD
True digestibility	96·16	5·58	96·35*	5·19
Biological value	64·03	2·68	65·58*	4·44
Net protein utilization	61·59	4·91	63·11*	4·69

Significantly different from period 1. * $P < 0.05$.

Table 8. *Effect of protein intake at 0·75 g/kg per d for 40 d on various blood indices in twelve young adult male Nigerians*

(Mean values and standard deviations)

	Initial		Period 1, 20 d		Period 2, 20 d	
	Mean	SD	Mean	SD	Mean	SD
Packed cell volume	0·48	0·026	0·47	0·032	0·47	0·0
Haemoglobin (g/l)	15·70	2·12	15·58	1·10	16·35	1·2
Blood sugar (mg/l)	836	112	817	81	816	90
Total protein (g/l)	74	5·9	71	5·1	72	5·0
Albumin (g/l)	41	4·8	38	5·2	38	4·1
Urea N (mg/l)	154	17·5	142	20·4	139	2·3
Creatinine (mg/l)	16	2·9	15	2·7	15	2·9
Cholesterol (mg/l)	1438	173·6	1388	167·3	1412	160
GPT (U/l)	10·04	1·99	12·57	3·67	12·00	3·3
GOT (U/l)	12·93	1·82	16·04	4·36	15·20	3·7

GPT, Alanine amino transferase; GOT, Aspartate amino transferase.
F, from analysis of variance; * $P > 0.05$.

Biochemical variables

The blood biochemical variables analysed are shown in Table 8. Statistical variables did not reveal any significant changes with time ($P > 0.05$). Howev some individual variations in certain blood variables. Blood urea-N was grea initial baseline period than during subsequent periods. The activities of the t serum alanine aminotransferase (*EC* 2 . 6 . 1 . 2) and aspartate aminotra 2 . 6 . 1 . 1), were relatively higher on the introduction of the diet but were time. The increase in the activities of transaminases could be accounted for b and M11 who were in negative N balance.

Packed cell volume, haemoglobin, blood sugar, total protein, albumin, cholesterol levels showed no significant changes.

DISCUSSION

The present investigation was not designed to determine the minimum protei for the adult male Nigerian, since only one level of protein intake was tested ascertain the adequacy of the previously estimated safe intake level of 0·7

Table 5. *Urinary creatinine excretion (mg/kg per d) of twelve adult Nigeria consuming their usual diet for 40 d (periods 1 and 2, each of 20 d)**

(Mean values and standard deviations)

Subject	Period 1		Period 2	
	Mean	SD	Mean	SD
M1	27·24	0·30	27·32	0·98
M2	25·00	0·90	24·44	0·24
M3	22·42	0·47	23·22	0·29
M4	25·50	0·36	25·47	0·28
M5	25·40	0·28	25·42	0·28
M6	27·19	0·40	27·57	0·13
M7	27·94	0·59	28·35	0·29
M8	26·94	0·28	26·26	0·31
M9	20·55	0·15	20·05	0·18
M10	22·97	0·22	23·45	0·63
M11	30·28	0·18	29·80	0·58
M12	26·69	0·09	26·72	0·17
All subjects	25·62	2·65	25·60NS	2·65

NS, not significantly different from period 1 ($P > 0·05$).
* Average of the last 5 d of each period.

Table 6. *Mean nitrogen intake, faecal N, apparent N absorbed (mg/kg body-wei and protein quality indices of habitual diets consumed by twelve adult Nigeria 40 d (periods 1 and 2, each of 20 d)*

(Mean values and standard deviations)

Period	N intake	Faecal N	Apparent N absorbed	Apparent digestibility	TD	BV
1 Mean	122·23	23·22	99·01	81·04	96·16	64·01
SD	12·14	7·06	11·19	5·29	5·58	2·68
2 Mean	122·84	22·97	99·82	81·04	97·35	65·58
SD	11·63	6·35	11·18	4·94	5·19	4·44

NS, not significantly different from values in group 1 ($P > 0·05$); TD, true digestibility; BV, biol NPU, net protein utilization.

Faecal output also did not show any significant changes with time. Table 6 apparent N absorbed as well as N digestibility. For the first and second exp periods, digestibility was calculated to be 81·04 and 81·31 % respectively.

Protein quality

The protein quality indices of the diets are shown in Table 7. The biological value net protein utilization (NPU) were calculated by the conventional methods obligatory N loss values of 69·23 mg N/kg body-weight per d as obtained from a study by Atinmo *et al.* (1985).

The digestibility values for periods 1 and 2 were 96·16 and 96·35 % respectively. BV values also showed no significant changes with time. BV and NPU values we 62 % respectively.

body-weight (Atinmo *et al.* 1981, 1988) with energy intake fixed at 0·2 MJ/kg boc per d. Although slight adjustments were made in the level of energy intake of so subjects during the first experimental period, body-weight showed no significant suggesting that energy intake was adequate during the study.

Urinary excretion did not fluctuate much with time. In fact, there was stabil urinary N excretion after an initial fall during the first 6 d of the experiment Subsequent variations were assumed to be due mainly to random and biological v (Rand *et al.* 1979). Urinary creatinine level was used as an index of the consist completeness of urine collections as well as an indicator of skeletal musculature 1970). Since the creatinine levels observed in the present study did not show any s changes, it may be said that no significant losses occurred in muscle or body ma subjects, thus implying an adequate adaptation to the level of protein in the die

Some of the principal difficulties commonly encountered in the interpretat balance values have centred around the uncertainties in estimating skin and misc N losses (Isaksson & Sjorgren, 1967; Calloway *et al.* 1971) and on the likel overestimating actual intakes and underestimating total N losses (Wallace, 19 cumulative errors that follow are likely to result in an overestimation of N retenti this background, special attention was paid to the measuring of N losses ar estimation of N in sweat. The N loss through this route was higher (10·4 mg N/ weight per d) than the value estimated by Atinmo *et al.* (1985) as obligatory N adult Nigerian men. In our opinion, the difference in values from those of Atin (1985) is most likely to be due to the higher N intake by subjects in the prese According to Cuthbertson & Guthrie (1934), an increase in N intake res corresponding increase in N excretion through sweat.

Faecal N output remained essentially the same throughout the course of the ex Faecal N can be affected by the composition or quality of the diet (Southgate, 198 present study, the quality and digestibilities (apparent and true) of the protein g high (81 and 95% for apparent and true digestibilities respectively). In additio and NPU were 65 and 62 respectively compared with those of egg protein (Hua 1982).

The mean N balance was positive during both experimental periods. All the pa except two, exhibited positive N balance. One of the two subjects, M11, lost weig end of the first experimental period. This was clearly reflected by a negative N ba also raised levels of aspartate aminotransferase and alanine aminotransamin energy intake of this subject was adjusted accordingly and by the end of th experimental period there were considerable changes, but not enough to enable th to attain a positive N balance. The positive N balance exhibited by the other subje the two experimental periods did not differ significantly. It is not, however, p determine from our findings, whether the observed positive N balance could dri balance with time, as theoretically, for adults, once N requirements are met an intake would normally cause a positive balance and finally, within a short perio return to zero balance. Reports by Oddoye & Margen (1979) from experiments they gave 36 g protein/d to six healthy young adults, however, showed no signific towards such an adaptation.

The difficulties and discrepancies associated with the N balance method ha many investigators to question its validity as the only criterion for estimating th of N which is necessary to maintain an individual in optimal health. Yoshimura (therefore suggested the use of other additional physiological indices. In the pres increases in concentrations of serum amino transferases (alanine and aspart observed in the first experimental period and a closer look at the values showed

was due mainly to the two subjects who had gone into negative N balanc however, fell with time and with an adjustment in the level of energy inta measurements of packed cell volume, haemoglobin, blood sugar, total pl albumin, urea, creatinine and cholesterol were within normal limits an significant changes with time.

The findings obtained from the present study point to the conclusion t intake of 0·75 g/kg body-weight per d with an energy intake of 0·2 MJ/kg per d was sufficient to maintain most of the subjects in positive N balance (+ body-weight per d) under normal physiological conditions. This observat clearly confirms earlier studies in adults, as well as in young adult Niger Atinmo *et al.* (1988).

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