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Assessment of Protein Needs of Nigeria Adult Males Using Short-Term Nitrogen Balance Technique

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Abstract: Protein requirement of Nigerian adult males aged 21-27 years from two different geographical locations in Nigeria was determined as a basis of comparison with previous studies. Eighteen young men from Northern and Southern Nigeria were recruited into the study and fed on a customary Nigerian mixed diet. The subjects were divided into dietary regimen groups of four protein levels (0.4, 0.7, 0.8 and 0.9 g protein/kg/day) at ordinary level of energy intake of 0.18 MJ/kg/day. The groups were assigned either an ascending or descending sequence of dietary changes. From regression analysis, the Nitrogen requirement for Nitrogen equilibrium was estimated to be 108.0±9.45 mg N/kg/day (0.68 g protein/kg/day) and 110.8±14.53 mg N/kg/day (0.69 g protein/kg/day) for the Northern and Southern groups respectively. Estimates for allowances to cover 97.5% of the population were 126.9 mg N/kg/day (0.79 g protein/kg/day) and 139.9 mg N/kg/day (0.87 g protein/kg/day) for the groups respectively. The mean Net Protein Utilization (NPU) for the diet was 64±1.29 with a true digestibility of 95.1±1.82. The requirement compared favorably and difference was not significant from previous studies. Thus, there is no need to set different requirements for any part of the country.

Key words: Protein requirement, N-equilibrium, Nigerian mixed diet

INTRODUCTION

The requirements of individuals and populations for protein and energy have been discussed extensively by experts and committees over the past few decades (Yarez *et al*., 1982; Morse *et al*., 2001; Elango *et al*., 2009). Although a rigorous definition for either protein or energy needs has not been unanimously agreed upon, various operational definitions have been formulated, and these have varied with time.

The current agreement indicates that the Recommended Dietary Allowance (RDA) for protein of 0.8 g of protein/kg/day safely and adequately meets the dietary needs of virtually all healthy people at or older than the age of 19 years (Council, 1989). There is also a general consensus that the protein requirement and suggested safe and adequate protein intake (i.e., protein allowance) of adults should be established primarily from shorter-term (2- to 3-week) nitrogen balance studies (Rand *et al*., 1977).

These recommendations are based on a meta-analysis of nitrogen balance studies (Rand *et al*., 2003), in which protein requirements are estimated by fitting a linear regression analysis model to the data and measuring zero nitrogen balance as the criterion of nutritional adequacy. However, the physiological response relationship between nitrogen intake and balance is not linear due to a decreased efficiency of protein utilization as zero balance is approached (Young *et al*., 1973; Rand and Young, 1999).

Conclusions from a very limited number of shorter-term nitrogen balance studies are conflicting; some support (Cheng *et al*., 1978; Zanni *et al*., 1979) and some question (Gersovitz *et al*., 1982; Bunker *et al*., 1987) the adequacy of 0.8 g of protein/kg/day for elderly people. Support for the conclusion that the RDA for protein may not adequately meet the dietary needs of many older people is found in a retrospective reanalysis of these shorter-term nitrogen balance data, based on calculations recommended by the 1985 Joint Food and Agriculture Organization, World Health Organization, and the United Nations University Expert Consultation (Council, 1989).

The question is ‘if this estimate will be adequate for all young adult males in Nigeria, irrespective of population groups of different ethnic backgrounds and environments’. It is a known fact that variation in protein requirements is the result of both growth potential and the modifying effect of the environment on the expression of such potential. It is therefore necessary to know the appropriate estimates for protein and energy requirement among different population groups in the country with different geographical environment, cultural background and consuming diets in physical characteristics. Also the capacity of the diets to meet

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protein requirements necessary to maintain adequate protein nutritional status under their prevailing environmental conditions should be determined.

This present study is aimed at determining the protein requirements of young adult Nigerian males living in different parts of the country with different geographical locations, dietary habits and culture.

MATERIALS AND METHODS

Subjects: Eighteen young men aged 21-27 years were recruited into the study. Seven of them were students of University of Maiduguri, Maiduguri (Northern Nigeria) designated M and the other eleven were from the University of Nigeria, Nsukka (South Eastern Nigeria) designated N. Characteristics of these are shown in Table 1.

The subjects were studied in their various campuses and subjected to their respective environmental conditions. Medical history, physical examination, as well as biochemical analysis of blood were obtained from each subject. Subjects certified as healthy were allowed to participate in the study. They maintained normal activity which included their full academic schedule and were free living but without hard physical exercise. Each student maintained a diary of all his activities through the study period.

To determine the energy intake needed to maintain the body weight under ordinary conditions, subjects were questioned in detail about their dietary intakes and physical activities prior to the Nitrogen balance studies. Each morning the subjects were weighed under the standardized conditions of preprandial, post-voiding and light indoor clothing.

Diet and experimental design: Food ingredients of the ordinary Nigerian mixed diets were carefully selected based on a combination of root tubers, cereals, vegetables and animal products which are common to the two groups studied, but cooked to the specification unique to respective areas. The basic ingredients and nutrient composition of the diet is shown in Table 2.

The average energy intake of all subjects was 0.18 MJ/kg/day. Complete mineral and vitamin supplements were added to prevent any deficiencies.

Subjects in each zone were divided into two groups; one group was assigned an ascending sequence of dietary changes while the other group has a descending sequence of dietary changes. Each dietary level was proceeded by one day on a practically protein free diet of less than 0.1 g protein/kg/day (Atinmo *et al*., 1985) to enhance adaptation, followed by 10 days on the test protein level. A break of three days between the different protein levels was allowed. Meals were consumed in patterns the subjects were accustomed to, i.e. 7-8 am, 1-2 pm and 7-8 pm under supervision.

Samples and analysis: Urine specimens were collected everyday on a 24 h basis in 2 litre plastic bottles containing 10 ml of 1N HCl. After measuring, an aliquot of each urine sample was frozen for analysis of total nitrogen. Faecal samples were collected with aid of a feal marker (carmine) as 5 day pools in pre-weighed plastic containers. Collections were held frozen prior to homogenization with a blender. The nitrogen content of the diets, urine and feaces were determined by the modified microkjedahl method of Munro and Fleck (1969).

For the last 5 days of each experimental period, N-balance was calculated from intake, fecal, urine and miscellaneous losses. Skin N loss was taken as 10.14 mg N/kg/day (Atinmo *et al*., 1988a).

Estimates of individual N requirements were by linear regression equation relating N intake to balance. The protein intake at which N balance was equilibrium was estimated as the requirement (Rand *et al*., 1977). Estimates of allowances for individual variations to cover the 97.5% population were taken as (+2 SD) of the mean (Rand *et al*., 1977).

The Biological Value (BV), Net Protein Utilization (NPU) and the true and apparent digestibilities for the mixed diet at different levels of N-intake in the young men were calculated by the conventional procedures of UNU (1979). Obligatory urinary, fecal and integumental Nitrogen losses were assumed to be 43.45, 18.32 and 7.46 mg.

Two way analysis of variance was used to test the level of significance between treatments, taking individual subjects as blocks. The student t-test was used to compare results between population groups.

RESULTS

Subjects: Changes in body weights and mid arm circumference of the subjects are presented in Table 1. The changes were not significantly ($p<0.05$) affected throughout the study period, among and within the two groups studied.

Nitrogen balance and requirements: Details of Nitrogen balance data are summarized in Table 3. An increase in urinary nitrogen was observed with increase in protein intake in both groups studied. All groups showed negative N-balance at 0.4 g protein/kg/day intake. More subjects reached N-balance with increase in dietary protein.

The regression equation obtained from individual data of the subjects who participated in the study is shown in Table 4. The protein requirements obtained for the Nigerian mixed diet for Maiduguri and Nsukka groups were 108.01±9.45 and 110.82±14.53 mg N/kg/day or 0.675 and 0.693g protein/kg/day respectively. The overall mean was calculated at 109.725±2.56 mg N/kg/day (0.686 g protein/kg/day) with an allowance of 0.843 g protein/kg/day.

Table 1: Characteristics of the young adult Nigerian male studied

Subject	Age (Years)	Weight (kg) Initial	Weight (kg) Final	Height (cm)	BMR* (MJ/day)	Mid arm circumference Initial	Mid arm circumference Final
Maiduguri							
M1	23	61.0	61.3	170.1	6.74	28.4	28.4
M2	26	58.0	58.6	171.0	6.55	29.0	29.0
M3	24	59.5	60.4	173.5	6.64	28.6	28.7
M4	23	52.3	52.3	170.6	6.18	24.7	24.5
M5	23	52.4	53.4	170.6	6.19	25.5	25.0
M6	22	49.7	49.6	166.3	6.02	26.1	25.4
M7	21	54.4	54.9	163.3	6.32	27.0	28.0
Nsukka							
N1	23	60.0	60.3	171.1	6.68	28.4	29.5
N2	22	56.3	54.9	167.6	6.44	26.0	26.5
N3	22	59.7	57.4	179.6	6.66	27.0	26.0
N4	25	68.3	68.3	170.6	7.21	29.6	30.0
N5	24	72.6	72.5	177.8	7.48	28.1	28.5
N6	23	59.4	59.4	168.5	6.64	27.1	27.0
N7	23	54.5	54.7	151.6	6.32	28.2	28.5
N8	27	61.9	61.6	171.6	6.80	28.0	28.0
N9	23	62.6	62.6	172.4	6.84	29.4	30.0
N10	24	58.2	57.8	162.1	6.56	27.8	26.5
N11	22	58.0	57.9	165.0	6.55	27.5	27.5
Mean	23.3	58.82	58.77+	169.37	6.60	27.58	27.61+
SD	1.50	5.55	5.52	6.02	0.36	1.35	1.61

*BMR is the Basal Metabolic Rate calculated from the equation BMR = 0.064 + 2.84 (FAO/UNU, 1985) where W = Weight; +No significant difference between final and initial values

Table 2: Ingredients and nutrient composition of the Nigerian mixed diet used in the study

Ingredients	Levels of protein intake (g/day) 0.4	0.7	0.8	0.9	Protein free
Custard flour	-	-	-	-	100
Corn meal	170	-	-	-	-
Bean cake	130	-	-	-	-
Refined sugar	25	100	100	50	20
Granted cassava	500	560	560	560	560
Onion	20	20	20	20	20
Pepper	2	2	2	2	2
Tomatoes	20	20	20	20	20
Red Palm oil	40	40	40	40	40
Okra	28	28	28	28	28
Boiled egg	-	56	56	56	-
Bread	-	100	100	125	-
Butter	-	28	28	28	-
Tea	Nil	Milk	-	-	-
Meat	-	40	60	80	-
Yam	-	400	400	400	-
Caloreen (glucose polymer)	100	-	-	-	200
Orange drink (0.5 MJ/bottle)	Varied among individuals				
Vitamin and Mineral supplements*				1 cap	
Nutrients					
Protein (g)	25.79	39.0	45.0	53.5	4.9
Energy (MJoule)	10.74	10.94	10.96	10.96	-

*The amount is for an average 59 kg subject. Vitamin and mineral was in the form of a capsule prepared by Ricker Laboratories. Langhborogh, England, UK

Protein utilization and digestibility: The Biological Value (BV), Net Protein Utilization (NPU) and true and apparent digestibilities for the Maiduguri and Nsukka groups are presented in Table 5. There were no significant differences ($p<0.005$) in the BV, NPU and true digestibility at different levels of protein intake in the same set of group. Also the differences observed in the mean parameter of 67.28 versus 67.23 for BV and 64.54

Table 3: Daily nitrogen data with diet (Mg N/kg/day)

Nitrogen intake	Urinary nitrogen	Feacal nitrogen	Total nitrogen loss*	Nitrogen balance
Maidugri!				
74.85±5.71	63.55±5.81	21.96±2.29	95.90±6.74	-21.04±7.07
112.84±9.14	73.83±7.44	24.16±3.54	108.39±6.69	2.85±8.54
130.03±9.66	82.14±7.44	22.80±1.99	115.13±8.94	14.90±10.22
156.23±12.17	92.45±7.83[ff]	22.80±2.07[ee]	125.66±7.56	30.57±14.02[ff]
Nsukka§				
63.92±5.39	57.87±11.04	23.98±1.95	92.25±12.05	-28.33±11.02
92.71±7.90	70.13±12.13	23.09±3.22	104.36±11.81	-11.64±9.86
116.79±9.78	75.55±13.31	23.79±2.60	109.76±12.31	7.04±11.34
147.69±12.07	90.73±14.15[ff]	24.36±1.94[ee]	125.50±12.41	22.20±13.01[ff]

*Total nitrogen loss include 10.41 mg N/kg/day for sweat Nitrogen (ref. 9). ! = Values are mean±SD (n = 7); § = Values are mean±SD (n = 11); ff = Significant difference from other levels of protein intake ($p<0.05$); ee = No significant difference from other levels of protein intake ($p<0.05$)

Table 4: Linear regression equations relating N-balance to N-intake for subjects

Subject	Regression equation*	Maintenance requirement	r	p
Maiduguri				
M1	Y = 0.527x-59.53	112.86	0.990	<0.05
M2	Y = 0.758x-79.02	104.24	0.992	<0.05
M3	Y = 0.553x-62.23	112.58	0.998	<0.05
M4	Y = 0.604x-71.61	118.63	0.989	<0.05
M5	Y = 0.677x-61.09	90.19	0.996	<0.05
M6	Y = 0.763x-86.61	113.56	0.995	<0.05
M7	Y = 0.563x-55.77	103.98	0.999	<0.05
Mean		108.01		
SD		9.45		
Nsukka				
N1	Y = 0.657x-85.48	130.04	0.991	<0.05
N2	Y = 0.740x-69.73	94.25	0.994	<0.05
N3	Y = 0.533x-68.48	128.61	0.986	<0.05
N4	Y = 0.322x-32.19	99.94	0.962	<0.05
N5	Y = 0.734x-72.41	98.68	0.938	<0.05
N6	Y = 0.946x-99.90	105.66	0.977	<0.05
N7	Y = 0.959x-70.00	106.28	0.976	<0.05
N8	Y = 0.586x-59.41	101.37	0.991	<0.05
N9	Y = 0.546x-53.65	98.19	0.949	<0.05
N10	Y = 0.563x-71.89	127.74	0.986	<0.05
N11	Y = 0.480x-61.56	128.23	0.975	<0.05
Mean		110.82		
SD		14.53		
Overall mean		109.73		
SD		12.56		

*Y = N-balance (mg/kg/day). x = N intake (mg/kg/day)

versus 63.39 for NPU of Maiduguri and Nsukka group respectively were not significant. The same applied to the 95.94 versus 94.32 for true digestibility.

Thus the overall values for the diet protein were 67.25±1.83 for BV and 63.96±1.29 for NPU. True digestibility was quite high at 95.13±1.82.

Values of apparent digestibility were somewhat lower at low protein intakes than at higher protein intakes ($p<0.05$) (Table 5) in both groups, although true digestibility did not differ.

DISCUSSION

Nicol and Phillips (1976) 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 background.

Thus the requirements in terms of protein for man are estimated to depend on a large extent on his ecological as well as socio-economic background. The customary diet plays an important role in terms of composition. Thus, our interest to investigate the extent of influence of ecological factors and customary diets on protein requirements in this particular study.

The experimental design was basically the recommendation of the United Nations University workshop (UNU Hunger program, 1979). Our subjects were from different geographical locations in the country but actually had a similar pattern as they were all resident university students. Thus, forming a good basis

Table 5: Biological Value (BV), Net Protein Utilization (NPU) and true and apparent digestibilities of the mixed Nigerian diet at different levels of protein intake

Level of protein intake (g/kg/day)	BV	NPU	Digestibilities	
			Apparent	True
Maiduguri*				
0.4	67.65±7.43ee	64.39±8.04ee	0.50±3.93ee	95.10±3.19ee
0.7	68.96±5.28ee	65.34±4.88ee	78.49±3.60ee	94.81±3.28ee
0.8	66.83±6.02ee	64.70±6.09ee	82.63±0.68ee	96.79±1.29ee
0.9	65.62±5.36ffee	63.71±5.45ffee	85.28±2.22§ee	97.07±1.50ffee
Nsukka!				
0.4	69.54±19.72ee	63.92±19.04ee	62.32±3.68ee	91.19±2.89ee
0.7	65.99±11.99ee	62.58±11.49ee	74.95±3.88ee	94.84±3.43ee
0.8	68.92±10.60ee	65.51±9.48ee	79.40±3.51ee	95.20±2.50ee
0.9	64.46±8.42ffee	61.84±7.46ffee	83.32±2.36§ee	96.07±1.47ffee

*Values are mean±SD (n = 7). ! = Values are mean±SD (n = 11); § = Significant difference from other levels of protein intake (p<0.05); ff = No significant difference from other levels of protein intake (p>0.05); ee = No significant difference between groups at same level (p>0.05)

of comparison with our previous studies at the University of Ibadan, Ibadan located in the south western part of Nigeria (Atinmo *et al.*, 1985;1988a,b).

Though it is extremely difficult to know if changes in the body weight as well as body composition occurred from day to day, yet maintenance of steady body weight is the accepted criterion for adequacy of energy intake (Rand *et al.*, 2003; Elango *et al.*, 2009).

Energy intake for weight maintenance varies with the protein intake. Therefore, the non significant changes observed in body weight and mid arm circumference of the subjects suggests that energy intake was adequate. The nitrogen loss in urine increased linearly with increase in protein intake as previously observed (Atinmo *et al.*, 1988b; Calloway and Margen, 1971; Young and Scrimshaw, 1968; Gersovitz *et al.*, 1982), while the fecal nitrogen was observed to be essentially the same irrespective of the level of protein intake. Thus, all subjects absorbed more nitrogen with increase in nitrogen intake. Therefore, the proportion of the intake absorbed (apparent digestibility) increased with increase in nitrogen intake.

In this study N-balance was taken up within the region of zero balance from our previous estimates (Atinmo *et al.*, 1988b) so as to evaluate critically the efficiency of utilization of the protein of the diet as opposed to over estimation with very low intakes.

Nevertheless, the paradox of a large nitrogen retention in normal subjects which is not reflected in the change in body weight has also posed a problem to this study. The assumption to this is the probability of an overestimation of nitrogen retention which could be attributed to many factors like the underestimation of total N-losses from actual skin and other miscellaneous losses. An estimated value based on similar totally different from the hotter drier Northern parts was used for the study (Atinmo *et al.*, 1988b). This value might not be adequate. Moreover, N-balance is dependent on other factors such as energy intake, physical activity, stress of any kind and the habitual intake (Milward *et al.*, 1989).

Net Protein Utilization (NPU) remained constant over the entire range of protein intake. The average value over the range was 64. This value was comparable to that in our previous studies (Atinmo *et al.*, 1988a;b) and also that of Huang and Lin (1982) on Chinese young men on a mixed diet.

Mean protein requirement of all individual subjects had a rather large coefficient of variation though they were exposed to minimal experimental variations. However the mean value of 108 mg N/kg/day obtained for the Maiduguri group was not significantly different (p<0.05) from that of the Nsukka group which was 110.82 mg N/kg/day. The safe level of N-intake taken as the mean requirements (+ 2 SD) which was expected to cover the needs of nearly 97.5% of the population was calculated to be 126.9 and 139.9 mg N/kg/day respectively for the Maiduguri and Nsukka group respectively. The mean requirement was taken as 134.85 mg N/kg/day (0.84 g protein/kg/day).

Conclusion: Though higher in value than our previous estimate, the requirements compare favorably and the difference was not significant. The lower estimate obtained in our previous study can be attributed to a higher energy intake.

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REFERENCES

Atinmo, T., G. Egun and C.M.F. Mbofung, 1988a. Long term evaluation of the adequacy of habitual diets to provide protein needs of adult Nigerian men. Br. J. Nutr., 60: 459-466.

Atinmo, T., C.M.F. Mbofung, G. Egun and B. Osotemehin, 1988b. Nitrogen balance study in young Nigerian adult males using four levels of protein intake. Br. J. Nutr., 60: 451-458.

Atinmo, T., C.M.F. Mbofung, M.A. Hussain and B.O. Osotemehin, 1985. Human protein requirements: Obligatory urinary and faecal nitrogen losses and the factorial estimation of protein needs of Nigerian male adults. Br. J. Nutr., 54: 605-611.

Bunker, V., M. Lawson, M. Stansfield and B. Clayton, 1987. Nitrogen balance studies in apparently healthy elderly people and those who are housebound. Br. J. Nutr., 57: 211-221.

Calloway, D.H. and S. Margen, 1971. Variation in endogenous nitrogen excretion and dietary nitrogen utilization as determinants of human protein requirement. J. Nutr., 101: 205-216.

Cheng, A., A. Gomez, J. Bergan, L. Tung-Ching, F. Monckeberg and C. Chichester, 1978. Comparative nitrogen balance study between young and aged adults using three levels of protein intake from a combination of wheat-soy milk mixture. Am. J. Clin. Nutr., 31: 12-22.

Council, N.R., 1989. Recommended Dietary Allowances. 10th Edn., Washington, DC: National Academy Press.

Elango, R., M.A. Humayuna, R.O. Ball and P.B. Pencharz, 2009. Evidence that protein requirements have been significantly underestimated. Curr. Opin. Clin. Nutr. Metab. Care, 12: 001-006.

Gersovitz, M., H. Munro, N. Scrimshaw and V. Young, 1982. Human protein requirements: assessment of the adequacy of the current recommended daily allowance for dietary protein in elderly men and women. Am. J. Clin. Nutr., 35: 6-14.

Huang, P.C. and C.P. Lin, 1982. Protein requirements of young Chinese male adults on ordinary Chinese mixed diet and egg diet at ordinary levels of energy intake. J. Nutr., 112: 897-907.

Milward, D.J., A.A. Jackson, G. Price and J.P.W. Rivers, 1989. Human amino acid and protein requirements: Current dilemmas and uncertainties. Nutr. Res. Rev., 2: 109-132.

Morse, M.H., M.D. Haub, W.J. Evans and W.W. Campbell, 2001. Protein requirement of elderly women: Nitrogen balance responses to three levels of protein intake. J. Geront. Med. Sci., 56A: M724-M730.

Munro, H.N. and A. Fleck, 1969. Analysis of urine and body fluids for nitrogenous constituents. In: Mounro, H.N. (Ed). Mammalian Protein Metabolism. Vol. 3. Academic Press, New York, pp: 423-535.

Nicol, B.M. and P.G. Phillips, 1976. The utilization of dietary protein by Nigerian men. Br. J. Nutr., 36: 337-351.

Rand, W.M., P.L. Pellett and V.R. Young, 2003. Meta-analysis of nitrogen balance studies for estimating protein requirements in healthy adults. Am. J. Clin. Nutr., 77: 109-127.

Rand, W.M., N.S. Scrimshaw and V.R. Young, 1977. Determination of protein allowances in human adults from nitrogen balance data. J. Clin. Nutr., 30: 1129-1134.

Rand, W.M. and V.R. Young, 1999. Statistical analysis of nitrogen balance data with reference to the lysine requirements in adults. J. Nutr., 129: 1920-1926.

United Nations University Hunger Programme, 1979. Protein energy requirements under conditions prevailing in developing countries. Current knowledge and research needs. The United Nations University, Tokyo.

Yarez, E., R. Uauy, D. Bal-Lester, G. Barrera, N. Chavez, E. Guzman, M.T. Saitua and I. Zacarias, 1982. Capacity of the Chilean mixed diet to meet the protein and energy requirements of young adult males. Br. J. Nutr., 41: 1-10.

Young, V.R. and N.S. Scrimshaw, 1968. Endogenous nitrogen metabolism and plasma free amino acids in young adults given a protein free diet. Br. J. Nutr., 22: 9-20.

Young, V.R., Y.S. Taylor, W.M. Rand and N.S. Scrimshaw, 1973. Protein requirements of man: efficiency of egg protein utilization at maintenance and sub maintenance levels in young men. J. Nutr., 103: 1164-1174.

Zanni, E., D. Calloway and A. Zezulka, 1979. Protein requirements of elderly men. J. Nutr., 109: 519-524.