A metabolic nitrogen balance study for 40 d and evaluation of the menstrual cycle on protein requirement in young Nigerian women

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(*Received 12 August 1991 – Accepted 14 October 1992*)

A long-term N balance study was carried out to determine the adequacy of an estimated protein requirement level recommended for young healthy Nigerian women and the effect of the menstrual cycle on the requirement. Eleven healthy young women, 25 (SD 2·6) years, were fed on a diet providing 0·6 g protein (N × 6·25)/kg per d and an average energy intake of 0·17 (SD 0·012) MJ/kg per d. Urine, faeces, sweat and menstrual fluids were collected for estimation of N balance. Menstrual N loss varied among individuals ranging from 46 to 124 mg N/d with an average of 89 (SD 21·8) mg N/d. Individual N balance was found to vary according to the day of the menstrual cycle. Positive N balances were recorded at about ovulation while negative balances were observed just before the onset of menstruation. The average N balance ranged from +8·49 (SD 5·64) to −430 (SD 7·84) mg N/kg per d. Nevertheless, an overall cumulative positive N balance of +5·7 (SD 6·98) mg N/kg per d which did not change significantly with time was observed for the last 5 d of two consecutive 20 d diet periods, although three subjects were in negative N balance. Blood biochemical measurements were stable except for one subject who had elevated serum aspartate aminotransferase (*EC* 2.6.1.1) levels. These findings suggest that our estimate of protein requirements was sufficient to achieve N balance equilibrium in a majority (70%) of young women. However, to satisfy 97·5% of the population, slight adjustments might be necessary in the energy intake since subjects who were in cumulative negative N balance also lost weight.

Protein requirement: Nitrogen balance: Menstrual cycle: Nigerian diet

Our previous estimate of protein requirement was derived from linear extrapolation of N equilibrium using data from short-term balance periods at several submaintenance levels of protein intake in the same group of subjects (Atinmo *et al.* 1988*b*; Egun & Atinmo, 1993).

The results of these studies may reflect the body's ability to adapt temporarily to inadequate protein intakes without giving a reliable indication of protein requirements for long-term maintenance (Yoshimura, 1972). More importantly with women, the effect of the menstrual cycle on N utilization cannot be fully evaluated and, thus, may lead to errors in estimating protein requirements (Calloway & Kurzer, 1982). Previous long-term studies (Calloway & Margen, 1971; Garza *et al.* 1977*a*; Gersovitz *et al.* 1982) have shown that short-term studies may not be adequate. Therefore, N balance studies of longer duration are desirable in order to assess whether there is adaptation to a given level of dietary protein intake. For these reasons, the long-term adequacy of our previous estimate of 0·6 g protein/kg per d (Egun & Atinmo, 1993) was assessed for 40 d. Also, the effect of the menstrual cycle on requirement was observed in our young women.

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MATERIALS AND METHODS

Subjects

Eleven female undergraduates of the University of Ibadan (Table 1) volunteered to participate in a 40 d N balance study to examine the effects of a diet supplying 0·6 g protein/kg per d, which was the protein requirement for university women in Nigeria determined in a short-term study (Egun & Atinmo, 1993). Their health status was evaluated on the basis of medical history, physical examination and routine laboratory tests. All subjects were studied under close supervision in their halls of residence. They continued their normal daily routines, including a full schedule of classes, but refrained from any unusual physical activity.

Daily body weights were recorded at 07.00 hours throughout the entire study period under standardized conditions (preprandial, post voiding and with light indoor clothing). Daily body temperatures were taken for determination of the day of ovulation (Hilgers & Bailey, 1980). The subjects kept a diary of their menstrual cycle. The study was approved by the Ethical Committee of the College of Medicine and subjects were required to sign consent forms.

Diet and experimental protocol

The experimental diet provided 0·6 g protein/kg per d and an energy intake ranging from 0·15 to 0·18 MJ/kg per d (Table 1). It was similar to the habitual diet of our previous study (Egun & Atinmo, 1993). The protein content of the diet was made up of about 500 g animal protein (beef) and 500 g plant protein/kg. Individual energy intake was calculated based on body weight, dietary history and careful monitoring of daily activities (Food and Agriculture Organization/World Health Organization/United Nations University (FAO/WHO/UNU), 1985). Predictive equations were used to calculate basal metabolic rate (BMR), which was used as the base for determining the energy requirement associated with activity (FAO/WHO/UNU, 1985). The diet was given in a meal pattern similar to that to which the subjects were accustomed, i.e. 07.00–08.00, 13.00–14.00 and 19.00–20.00 h. Subjects were required to adhere strictly to the experimental diet. Nutrient intakes were maintained throughout the entire period.

For the purpose of exploring adaptation to the test protein the 40 d were arranged into two consecutive 20 d diet periods. For N balance studies the metabolic periods were taken as days 16–20 and 36–40. Also, N balances were determined on individuals at the time of their ovulation and before the onset of menstruation.

Samples and measurements

Complete 24 h urine samples were collected throughout the study. Faecal samples were collected during the two 5 d metabolic periods with carmine marker. Faecal samples were collected daily, pooled and homogenized. Menstrual losses were collected using the method of Calloway & Kurzer (1982) on preweighed sanitary towels or tampons and N extracted with 0·1 M-HCl for 24 h. Sweat loss was collected as before (Egun & Atinmo, 1993) on clothes made N-free, for 2 d during the metabolic periods.

Total N, urea and creatinine in urine, and N in faeces, menstrual extract, sweat and diet were measured as described previously (Egun & Atinmo, 1993).

Venous blood samples were obtained after an overnight fast at the beginning, middle and end of the study. Whole blood was taken for estimating packed cell volume, haemoglobin and glucose. Total serum proteins, albumin, globulin, urea, creatinine, aspartate and alanine aminotransferases (*EC* 2.6.1.1 and 2.6.1.2), alkaline phosphatase (EC 3.1.3.1), cholesterol, uric acid, triacylglycerols and fatty acids were determined by standard

Table 1. *Physical characteristics of young Nigerian women studied for 40 d and their overall weight change*

Subject	Age (years)	Height (m)	Initial body wt (kg)	Energy intake (MJ/kg per d)	BMR* MJ/d	Wt change during study (kg)
F_1	21	1·68	70·5	0·16	6·64	−2·5
F_2	26	1·57	50·0	0·17	5·17	+0·5
F_3	27	1·69	63·5	0·15	6·00	−2·0
F_4	24	1·56	56·0	0·17	5·54	+1·0
F_5	24	1·64	61·5	0·15	5·88	−1·5
F_6	27	1·55	51·5	0·18	5·26	0·0
F_7	26	1·54	54·0	0·18	5·41	+0·5
F_8	30	1·50	49·0	0·18	5·12	0·0
F_9	23	1·60	57·5	0·16	5·63	+0·5
F_{10}	24	1·67	52·5	0·18	5·32	−1·0
F_{11}	22	1·59	53·5	0·18	5·38	+1·0
Mean	25·0	1·60	56·3	0·17	5·58	−0·32
SD	2·60	0·06	6·50	0·01	0·45	1·23

BMR, basal metabolic rate.
* Calculated from Food and Agriculture Organization/World Health Organization/United Nations University (1985).

biochemical methods. Anthropometric measurements were made to detect possible changes in body composition. These measurements included mid-arm circumference and skinfold thickness at the triceps, biceps, subscapular and supra iliac regions.

Statistical analysis

N balance (mg N/kg per d) was calculated from the analysed N intake, daily urine total N for the last 5 d and daily faecal N (estimated from 5 d faecal pool). In addition, miscellaneous N losses through sweat and menstruation were included.

Significance of changes in the N balance response to the experimental diet was determined by paired *t* test. Evaluation of changes in selected biochemical variables were determined by two-way analysis of variance using individual subjects and metabolic periods (days 16–20 *v.* 36–40) as independent variables. The dependent variables were the biochemical variables determined before beginning the diet and after each of the two 20 d intervals. Probability of $P < 0·05$ was accepted as significant.

RESULTS

Body-weight changes for each individual for the entire 40 d experimental period are presented as part of Table 1. A fall in weight to the level of 0·32 (SD 1·23) kg (CI 1·86–2·60) was observed among all the subjects. When viewed on an individual basis, the fall can be attributed mainly to subjects F1 and F3. Subject F1 lost a considerable amount of weight (2·5 kg) throughout the study. The difference in body weight between the initial body weight and the two diet intervals were not significant ($P > 0·05$): Table 2). However, significant differences were observed among individuals ($F_{3,10}$ 180·8).

Anthropometric measurements of mid-arm muscle circumference, waist circumference, total skinfold thickness and percentage body fat were slightly but not significantly reduced with time (Table 2).

Table 3 provides the N balance data for young adult females and also the urea and

Table 2. *Anthropometric measurements of young Nigerian women studied for 40 d**
(Mean values and standard deviations for eleven subjects)

	Initial		First 20 d period		Second 20 d period		
	Mean	SD	Mean	SD	Mean	SD	*F*†
Wt (kg)	56·3	6·5	56·0	5·7	56·0	5·6	0·64
Mid-arm muscle circumference (mm)	253	14	254	10	250	12	0·90
Waist circumference (mm)	689	32	690	38	687	27	1·54
Total skinfold thickness (mm)	68·8	13·0	68·6	13·9	68·4	12·2	0·43
Percentage body fat	23·0	2·7	23·2	3·0	22·8	2·6	1·80

* For details of subjects and procedures, see Table 1 and pp. 450–451.
† There was no significant difference with time (two-way ANOVA for $df_{3,33}$).

Table 3. *Nitrogen balance (mg/kg per d) for young Nigerian women given 0·6 g protein/kg per d for 40 d*†

	First 20 d period							Second 20 d period						
						UCr‡							UCr‡	
Subject	NI	UN‡	FN§	NB‖	UUN‡	Mean	SD	NI	UN‡	FN§	NB‖	UUN‡	Mean	SD
F_1	77·9	57·2	16·7	−7·3	41·8	18·4	0·48	79·1	55·6	16·4	−4·2	41·7	18·6	0·17
F_2	106·0	66·6	17·1	+11·0	50·7	22·9	0·17	105·0	64·1	17·6	12·0	50·9	21·6	0·49
F_3	85·5	61·2	16·4	−3·4	44·5	21·7	0·21	85·5	65·7	16·9	−8·4	48·8	21·0	0·24
F_4	97·3	64·1	16·9	+5·0	46·5	20·9	0·49	96·4	61·8	16·3	+7·0	44·5	19·9	0·19
F_5	86·9	60·1	17·7	−2·0	42·6	21·9	0·17	87·6	63·4	20·9	−8·0	44·6	21·3	0·11
F_6	101·9	63·8	17·2	+9·6	47·4	18·7	0·13	102·9	65·6	17·8	+8·2	48·7	18·7	0·11
F_7	100·0	58·6	17·0	+13·1	43·5	19·2	0·38	100·0	58·2	18·2	+12·3	42·7	18·3	0·15
F_8	107·1	67·0	16·6	+12·2	50·0	18·2	0·32	108·2	64·4	17·8	+14·7	48·1	17·6	0·17
F_9	89·8	56·1	17·4	+5·0	40·7	18·9	0·12	91·4	56·2	16·5	+7·4	43·4	18·9	0·12
F_{10}	102·9	61·7	18·8	+11·1	45·6	18·7	0·13	103·9	61·7	18·6	+12·3	44·5	18·7	0·09
F_{11}	99·1	57·6	22·2	+8·2	41·9	18·4	0·2	101·9	57·1	22·8	+10·7	42·0	17·8	0·06
Mean	95·6	61·3	17·6	+5·7	45·0	19·8	—	96·5	61·3	18·2	+5·8	45·5	19·3	*
SD	9·44	3·76	1·59	6·98	3·4	1·7	—	9·4	3·81	2·02	8·52	3·14	1·42	—

NI, N intake; UN, urinary N loss; FN, faecal N loss; NB, estimated true N balance; UUN, urinary urea-N; UCr daily creatinine excretion (g/d).
Mean values are significantly different from that for first 20 d period: * $P < 0.05$.
† For details of subjects and procedures, see Table 1 and pp. 450–451.
‡ Mean for last 5 d of each period.
§ Determined from 5 d faecal pool.
‖ Including integumental N losses measured at 9·7 mg N/kg per d and menstrual N loss at 1·6 mg N/kg per d.

creatinine excretion, all measured during the last 5 d of each 20 d period. N balance was calculated with integumental and menstrual N losses measured at 9·7 (SD 0·44) and 1·6 (SD 0·35) mg N/kg body weight per d respectively. The N balance for the subjects during the successive two 20 d intervals were (mg N/kg per d) +5·65 (SD 6·98) and +5·82 (SD 8·52) respectively and not significantly different ($P > 0.05$). Three subjects were in negative N balance during the two successive dietary intervals. Subject F1 had the most negative

Table 4. *Apparent nitrogen absorption (mg/kg body weight per d), digestibility and protein quality indices of habitual diets consumed by young Nigerian women for 40 d*

(Mean values and standard deviations for eleven subjects)

	First 20 d period		Second 20 d period		
	Mean	SD	Mean	SD	t_{10}*
N intake (mg/kg per d)	95·9	9·44	96·5	9·35	—
Faecal N (mg/kg per d)	17·6	1·59	18·2	2·02	1·64
Apparent N absorbed (mg/kg per d)	78·2	9·25	78·4	9·05	0·207
Apparent digestibility (%)	81·5	2·18	81·1	2·45	1·13
True digestibility (%)	97·5	1·58	96·9	2·06	1·42
Biological value (BV)†	0·494	0·033	0·485	0·056	0·75
Net protein utilization (NPU)†	0·48	0·32	0·49	0·055	0·82

* All values were not significantly different ($P > 0.05$) from first 20 d period (paired t-test).

† BV and NPU were calculated by the conventional methods (obligatory losses were taken as 40·00 mg N/kg per d).

balance during the first 20 d interval but the balance improved by the second interval because her energy intake was stepped up when she lost a considerable weight by the end of the first interval. With time, subjects F3 and F5 showed increased negativity in N balance.

Urinary creatinine, which was used as an index of the consistency and completeness of urine collections, was observed to be significantly reduced with time ($P < 0.05$). Urinary N excretion, however, did not change with time (Table 3). Also the 74% contribution of urinary urea-N to total N was unchanged throughout the study ($P > 0.05$).

Faecal N output did not show significant changes with time-period on the diet. Table 4 shows the apparent N absorbed as well as N digestibility. For the first and second dietary interval the digestibility was calculated to be 81·5 (SD 2·18) and 81·1 (SD 2·45) respectively.

The protein quality indices of the diet (Table 4) showed that the biological value (BV) and net protein utilization (NPU) were not affected with time. BV and NPU were approximately 0·49 and 0·475 respectively. True digestibility was quite high at 97%.

To check the effect of the day of the menstrual cycle on N metabolism, N balance was performed for all subjects at periods around ovulation and just before the onset of the next menstrual flow.

Menstrual cycles in subjects averaged approximately 28 (SD 0·5) d. Ovulation was judged to be approximately midway in each cycle from the slight rise in daily body temperature observed for most subjects. Throughout the entire study, at least two menstrual collections were made. Menstrual N loss is shown on Table 5. This varied considerably among individuals and ranged from 49 to 120·5 mg/d with an average of 89·0 (SD 21·5) mg/d (N loss in menses divided by duration of loss).

N balance between days 13 and 17 after menstruation (ovulation) and days 5 and 10 before the onset of the next menstruation (i.e. days 19–24) was determined for individual subjects (Table 5).

A positive N balance was recorded around ovulation, but just before the onset of menstruation there was a significant reduction in retention of N and almost all subjects were in negative N balance (Table 5). Balance was greatly influenced by the urinary N excretion at these times because faecal N loss used for balance studies was taken as 1·79 mg N/kg per d (the average of all faecal N losses).

Table 5. *Menstrual cycles, menstrual nitrogen losses and nitrogen balance in young Nigerian women*†

Subject	Length of cycle (d)	Duration of menstruation (d)	Menstrual N loss			N balance (mg/kg per d)	
			g	mg/d	mg/kg per d	Ovulation	Before menstruation
F_1	28	5	3·38	120·5	1·79	+1·66	−17·73
F_2	28	6	1·35	49·0	0·99	+18·7	+6·10
F_3	28	7	2·73	97·4	1·54	+0·70	−10·31
F_4	27	5	2·04	75·5	1·39	+6·10	−1·13
F_5	28	6	2·57	91·7	1·52	+3·53	−10·92
F_6	28	5	2·67	100·9	1·91	+13·30	−0·73
F_7	27	7	2·60	92·8	1·75	+14·34	+1·43
F_8	28	6	2·70	104·7	2·12	+7·65	−6·19
F_9	28	5	3·04	110·1	1·90	+10·60	+7·04
F_{10}	28	7	2·00	71·7	1·41	+9·69	−9·64
F_{11}	27	6	1·74	64·5	1·24	+7·69	−7·53
Mean	27·7	5·9	2·46	89·0	1·6	+8·54	−4·51**
SD	0·5	0·8	0·61	21·5	0·32	5·54	7·71

Balance at the onset of menstruation was significantly different from that at ovulation ** $P < 0·01$ (t_{10} 9·32, df_{10}).

† For details of subjects and procedures, see Table 1 and pp. 450–451.

Results of the various assays of blood are summarized in Table 6. The mean values of all blood variables measured did not show significant changes with time ($P > 0·05$). However, the mean activity of aspartate aminotransferase tended to increase with time. This rise was attributed to the result of subject F5, who had an overall cumulative negative N balance. The enzyme rose to an abnormal level by the end of the study.

DISCUSSION

The protein intake in the present study was the value estimated from our previous study as the requirement for adult females of the University. This allowance of 0·6 g protein/kg per d was fed along with an energy intake of 0·15–0·18 MJ/kg per d, according to individuals' requirements, for a 40 d period to ascertain its adequacy.

Individual body weight response showed that there was a general loss in body weight of about 0·36 (SD 1·23) kg. Most of the weight loss was in the first 20 d of the study and a closer look at findings showed that two of the subjects F1 and F3 lost considerable body weight of 2·5 and 2·0 kg respectively. Energy intake was increased in these subjects and their weights increased. However, some of the other subjects lost weight initially but were stable by the end of the study. There seems to be a need for a slight increase in energy intake among most subjects. But this needs caution as it is known that excess energy intake would overestimate N retention (Calloway & Spector, 1954). The non-significant changes observed with all anthropometric measurements support the suggestion that energy intakes were only marginally adequate (Miller & Mumford, 1967).

N output was measured as precisely as possible to avoid underestimation. N losses through skin (sweat) and menstruation were carefully estimated.

Recommended allowances for integumental losses have been found to be too low for tropical climates (Huang *et al.* 1975), and could give an erroneous N balance. Faecal N was not affected by time, and excretion changed little throughout the course of the experiment.

Table 6. *Effect of a 40 d period at 0·6 g protein/kg per d on various blood biochemical variables in young Nigerian women*

	Initial		First 20 d period		Second 20 d period		Statistical significance of difference between periods	
	Mean	SD	Mean	SD	Mean	SD	*F*	
Packed cell volume	0·41	0·02	0·40	0·02	0·40	0·02	0·207	NS
Haemoglobin (g/l)	132	16·2	130	10·1	130	12·8	2·033	NS
Blood Glucose (g/l)	791	120·0	803	95·0	801	97·1	0·780	NS
Total protein (g/l)	70	7·2	70	6·6	70	8·9	0·039	NS
Albumin (g/l)	39	4·5	37	3·0	37	4·2	1·288	NS
Globulin (g/l)	31	8·5	34	8·5	33	8·5	1·403	NS
Urea nitrogen (g/l)	145	25·4	135	31	131	22·4	2·323	NS
Creatinine (mg/l)	16	4·2	16	3·1	15	2·9	1·385	NS
Uric acid (mg/l)	43	8·6	43	8·6	43	8·8	0·085	NS
Triacylglycerol (mg/l)	862	112·6	855	77·1	854	79·3	0·290	NS
Cholesterol (mg/l)	1792	356	1750	290·1	1756	49	0·184	NS
Aspartate aminotransferase (EC 2.6.1.1; U/l)	16·3	6·57	18·3	8·26	21·6	9·11	0·830	NS
Alanine aminotransferase (EC 2.6.1.2; U/l)	12·7	3·09	12·0	2·54	12·7	3·47	0·218	NS
Alkaline phosphatase (EC 3.1.3.1; U/l)	48·7	13·36	50·4	12·75	51·4	12·93	0·424	NS

NS, not significant ($P > 0·05$; two-way ANOVA).

The digestibilities of the experimental diet in terms of protein (apparent and true) were quite high at 81 and 95% respectively, which is similar to values obtained in our earlier study on young men fed on the same diet (Atinmo *et al.* 1988*a*). However, the protein quality indices differed. Our female subjects were observed to have lower values for BV and NPU. The BV and NPU were 0·49 and 0·48 respectively compared with 0·65 and 0·62 for our men. The reason for this difference in protein utilization is not clear but may be attributed to hormonal interference (Calloway & Kurzer, 1982).

The N balance data from our subjects showed that on a protein level of 0·6 g/kg per d body N equilibrium was achieved in about 70% of the subjects. Three of the eleven subjects were in negative N balance though the mean N balance, based on the last 5 d of each 20 d interval, was positive.

However, the total urinary creatinine excretion data suggest greater cumulative body protein losses than indicated by the balance data. The urinary creatinine excretion showed significant reduction with time and, thus, tends to indicate greater losses of muscle mass than did the cumulative N balance. Similar results were obtained for the studies of Garza *et al.* (1977*a*). They observed decreases in creatinine excretion in all their subjects who had a cumulative negative N balance. Although Crim *et al.* (1975) also observed a progressive fall in creatinine excretion with time at a constant level of protein, their diet was creatine–creatinine free.

Calloway & Kurzer (1982) suggested that hormonal effects influenced N utilization at different times of the menstrual cycle. Menstrual loss varied between individuals and our value (89 mg N/d) was much higher than values (43 (SD 24) and 67 mg N/d) reported by Calloway & Kurzer (1982) and Hallberg *et al.* (1966) respectively. N balances observed at about ovulation were positive but fell significantly just before the onset of menstruation. This pattern was in agreement with the results of Calloway & Kurzer (1982). It may be suggested that hormonal influences lead to rhythmic gain and loss of soft tissue N with N being retained more avidly during the luteal phase. This observation complicates N balance studies on women and for meaningful recommendations based on N balances the duration of the study should be extended to cover the entire menstrual cycle.

According to Yoshimura (1972) it is necessary to test the functional costs of tolerance to the level of protein intakes. Measurements of packed cell volume, haemoglobin, serum protein, albumin, globulin, urea, uric acid, creatinine, triacylglycerols, cholesterol, aspartate and alanine aminotransferases and alkaline phosphatase did not show significant changes with time. However, aspartate aminotransferase showed an increase in activity. The individual data showed that the increase was attributed mostly to subject F5 who had an abnormal increase and an overall negative N balance. Similar abnormal increases in serum transferase activity have been observed by Garza *et al.* (1977*b*) in their subjects.

Although we observed a cumulative positive N balance and the intake was sufficient for eight of our eleven subjects, it is still necessary to make adjustments in our estimated requirements to cover 97·5% of the population. It may be that the major adjustment should be in the energy intake, since subjects who had lost weight showed improved balance when their energy intake was increased. Thus, there is the need to estimate the energy requirements of these women directly. In the present study the energy needs of individuals were estimated from calculations of BMR based on predictive equations (FAO/WHO/UNU, 1985). These equations may not necessarily be applicable to all populations, especially in the tropics.

The authors are grateful to the United Nations University, University of Ibadan and Nigerian Institute of Social and Economic Research (NISER) for the grants provided for this work. The authors wish to thank Professors Nevin Scrimshaw and Doris Calloway for their advice and critical evaluation of the manuscript. They are grateful for the full cooperation of the subjects in the study.

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Printed in Great Britain