NUTRITION RESEARCH, Vol. 12, pp. 199-208,1992
0271-5317/92 $5.00 + .00 Printed in the USA.

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SWEAT NITROGEN LOSSES OF YOUNG ADULT FEMALES IN NIGERIA WITH DIFFERENT LEVELS OF DIETARY PROTEIN INTAKE.

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ABSTRACT

Nitrogen balance studies were carried out in two separate experiments involving a total of fifteen acclimatized young women. The aim was to determine the effects of different levels of protein intake on sweat nitrogen losses. The losses were determined through the collection of 24 hour total body sweat samples under controlled environmental conditions. In nine of the subjects fed on graded level of protein for a ten-day period at a time, sweat nitrogen losses varied with nitrogen intake, being 5.45±0.79, 6.36±0.70, 6.65±0.73 and 7.10±0.87mg N/kg/day at protein intakes of 0.3, 0.4, 0.5 and 0.6 protein/kg/day, respectively. Obligatory nitrogen loss in sweat extrapolated from linear regression analysis of nitrogen intake versus sweat nitrogen loss was 4.20 mg N/kg/day. When the subjects were fed a single level of protein (0.6 g/kg/day) for forty days, the mean sweat nitrogen loss of the women was 9.45±0.44 mg/kg/day. Thus the recommended allowance for sweat nitrogen losses (3-4 mg N/kg/day) in estimating protein requirement by the 1985 FAO/WHO/UNU expert report is an underestimation under conditions prevailing in tropical countries.

Key words: Sweat Nitrogen, Protein Intake, Nitrogen Balance.

INTRODUCTION

Most studies undertaken to assess protein requirements have either excluded protein needed for the integumental nitrogen losses as negligible and unlikely to be affected by diet (1) or have used 3-4 mg N/kg/day for sweat nitrogen losses as suggested by the 1985 FAO/WHO/UNU report (2). The estimate used in the FAO/WHO/UNU report was derived mainly from studies conducted under moderate ambient temperatures and correspond to obligatory losses (3). The applicability of this figure for N-balance studies conducted under conditions of high environmental temperatures such as exists in the tropics and at protein intakes in the submaintenance to maintenance range has been questioned, since increased sweating and nitrogen intake affect skin losses (4-6). Furthermore, loss of nitrogen via one route may be compensated for by changes in the loss of nitrogen via another. A compensatory reduction of urinary nitrogen when sweat nitrogen loss increased has been observed by many

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investigators (7-9). Therefore, unless all routes of nitrogen losses are measured, erronous conclusions may be drawn from apparent nitrogen balance and in consequence requirements underestimated.

The purpose of this study was to estimate the sweat nitrogen losses of subjects living under tropical climatic conditions where increased sweating may alter total nitrogen needs. University students were studied, as a basis of comparison with similar studies elsewhere (3,4 and 9). Also they provide an average estimate for sweat loss under conditions prevailing in developing countries, as their activity pattern is moderate.

METHODS AND MATERIALS

Subjects

Fifteen young women all physically healthy participated in the study. The subjects were all fully acclimatized to a high environmental temperature. They lived under close supervision in their hall of residence and engaged in normal routine daily activities which included their full academic schedule, while maintaining a reasonable constant level of physical activity during the study periods.

The subjects were aged between 21 and 32 years. Their body weights and heights ranged between 45 to 70 kg and 154 to 170 cm, respectively. Body surface estimated from a nomograph was between 1.40 and 1.80 m^2. Body weight was measured daily before breakfast in order to assess the adequacy of caloric intake.

The environmental conditions were uncontrolled while temperature and relative humidity were recorded at a nearby experimental station. From the readings provided, average values were recorded for periods of sweat collection.

Diet

The diets were standardized based on foods habitually consumed and given in meal patterns which the subjects were accustomed to, that is, 7-8 am, 1-2 pm and 7-8 pm. Throughout the study, all subjects were required to adhere to the experimental diet and eat only the food prepared by the investigators. Table 1 shows the composition of the diet. Adjustments in the protein intake were made by increasing the dietary beef intake. Complete vitamins or minerals were lacking. Energy intake for individual subject was kept constant throughout the entire study periods.

Experimental Design

Two nitrogen balance studies were carried out. the first experiment (Expt. 1) was based on short-term feeding of graded levels of protein at 0.3, 0.4, 0.5 and 0.6 g protein/kg/day for a period of 10 days per protein level. The second experiment (Expt. 2) was a 40-day continuous feeding of a single level of protein.

TABLE 1

Composition of Diet, Ingredients and Nutrients

Ingredients	(g)
Bread	90
Refined Cane Sugar	30
Margarine	30
Pepper (dried)	4
Tomatoes (fresh)	80
Onions	40
Palmoil	60
Cassava (grated)	100
Beef	10
Vegetables	14
Yam (cooked)	200
Orange flavoured drinks* (bottles)	(2)
Nutrients: Protein+ (g)	15.68
Energy (kcal)	2168.35

* 1 botle contains 120 kcal.
\+ Lowest protein intake in the study (providing 0.3 g protein/kg/day)

In experiment 1, nine women participated and the study lasted 56 days consisting of four experimental diet periods each of 10 days duration in which the four different dietary levels of protein were fed. The experiment followed the standard protocol for short-term nitrogen balance studies with graded levels of protein as previously described (10).

In experiment 2, the six subjects were fed 0.6 g protein/kg/day for 40 days continuously. This level of protein is an estimated dietary allowance for young university women in Nigeria. The 40 days were arranged into two consecutive 20 day diet periods. The metabolic periods were taken as days 16-20 and 36-40.

Sample and Analysis

Skin nitrogen loss through sweat was collected for two days at the end of the metabolic period of each experimental dietary level. Sweat was collected by the total body method similar to techniques described by Spence, Abernathy and Ritchey (11) and Sirbu, Margen and Calloway (12) with slight modifications.

Prior to each period of sweat colection, all towels, washed clothes, beddings and clothings to be used by the subjects including their underwears on individual basis were soaked overnight in 0.5% acetic acid and rinsed six times in distilled deionized water to remove any trapped nitrogen to ensure proper estimation of the loss accountable to sweat during the collection periods. Colour fast clothings were chosen to minimize nitrogen contribution from dyes and to maximize absorption of sweat. At the onset of collection, subjects washed their bodies thoroughly, rinsed with distilled water and towel dried vigorously with a nitrogen free towel. The bath water as well as the towel were discarded. The subjects were dressed in their nitrogen-free clothings consisting of underwear and three-quarter length cotton dresses.

Each subject was given a marked towel (wash cloth) which was used to dry any visible sweat from her face, neck, arm and legs during the collection period. Subjects were cautioned against contaminating their underwear with urine and faeces. The subjects wore the same clothing for two days. At the end of this period, the subjects removed all the clothing and bathed thoroughly by scrubbing without soap (so as not to contribute any unknown source of nitrogen) in specified amounts of deionized distilled water, about 20-30 litres, and then towel dried vigorously. A sample of bath water was saved in thoroughly rinsed plastic sample bottle with distilled-deionized water and stored at $-20^{o}C$ until analysed for nitrogen.

The sweat and skin losses were extracted from the clothings, wash cloths, beddings and towels by soaking them in specified volumes of 0.05% acetic acid solution for 48 hours, and a sample of the soaking solution taken in individual labelled plastic containers and stored at $-20^{o}C$ until analysed. This method assumes the garments and soaking solution to be at equilibrium. A similar study by Calloway et al. (3) showed that conventional washing and rinsing procedures did not completely remove the nitrogen from the cloth. However, the difference in amount of nitrogen recovered after repeated rinsing was within the limits of accepted error (+ 10%).

Urine was collected for each 24 hour period and faeces, which were marked with carmine were pooled for 4 to 5 day periods. Urine was analysed for nitrogen and creatinine (which was used to judge the completeness of urine collection) and the nitrogen content of the faeces were determined.

The nitrogen content of sweat collection, urine, faeces and food samples were analysed using the modified Kjeldahl-Cunning-Arnold method with copper and selenium catalysts (13).

Blood samples were taken before breakfast at the beginning and end of each metabolic period. Plasma was analysed for urea nitrogen by the manual carbaminodiacetyl reaction of Marsh, Fingerhust and Miller (14).

RESULTS

In experiment 1, total nitrogen loss through the skin averaged 5.45 ± 0.79, 6.36 ± 0.70, 6.65 ± 0.73 and 7.10 ± 0.87 mg N/kg/day on protein intakes of 0.3, 0.4, 0.5 and 0.6 g protein/kg/day, respectively. Table 2 shows the mean total nitrogen loss in skin as well as the nitrogen content of each fraction of sweat collection. It was observed that the nitrogen adhering to clothes was much larger than the nitrogen retained on the skin. Clothes nitrogen was 71% of total sweat collection.

TABLE 2

Nitrogen Content* of Fractions Analysed and Total Skin Loss in Young Women.

Component	Level of Protein Intake (g/kg/day) 0.3	0.4	0.5	0.6
Clothes	3.89±0.63	4.50±0.64	4.76±0.60	5.10±0.69
Body	1.59±0.19	1.86±0.23	1.89±0.15	2.00±0.21
Total skin loss	5.45±0.79	6.36±0.70	6.65±0.73	7.10±0.87+

* Expressed as mean ± SD of nine subjects (in mg N/kg/day).
\+ Significant differences in changes with levels of protein intake at $p < 0.05$ (2-way ANOVA).

The mean sweat nitrogen losses (skin) expressed per 24 hours and per square metre of body surface per 24 hours increased as the level of dietary protein increased (Table 3). Individual subjects varied somewhat in mean sweat nitrogen values, but differences in mean sweat nitrogen losses, as determined by analysis of variance (2-way ANOVA) were significant at $p < 0.05$. Individual variation in sweat nitrogen values can be attributed to variations in activity and individual characteristics.

Increase in nitrogen intake resulted in a corresponding increase in skin excretion of nitrogen. The correlation was positive and significant ($r = +0.97$, $p < 0.05$). The mean regression equation of individual subjects is represented as $Y = 4.2 + 0.03X$, where Y is skin nitrogen loss in mg N/kg/day and X is nitrogen intake in mg/kg/day. The intercept of the regression when intake was zero was 4.2 mg N/kg/day. This was taken as the obligatory nitrogen loss through the skin.

TABLE 3

Dietary Nitrogen Intake, Skin Nitrogen Loss and Plasma Urea Nitrogen in Young Women*.

Nitrogen Intake g/day	Skin Nitrogen Loss mg/day	mg/m^2/day	Plasma Urea Nitrogen mg/100ml
2.51	286.0±74.1	182.3±39.1	6.09±0.76+
3.42	327.8±70.0	209.5±37.2	6.72±0.70+
4.38	349.5±71.5	222.9±35.4	7.54±1.06+
5.23	370.7±84.1	236.4±42.0	8.29±0.95+

* Values are expressed as mean ± SD of nine subjects.
\+ Mean values were significantly different from the other values in the column (2-way ANOVA) : $p < 0.05$.

Skin nitrogen loss increased with increase in plasma urea nitrogen (Table 3). A correlation of the two showed a positive significant relationship ($r = +0.96$, $p < 0.05$). Plasma urea nitrogen increased significantly with increase in plasma intake ($p < 0.05$ with 2-way ANOVA).

Environmental conditions were fairly constant. The temperature and relative humidity were between 25.5 and 26.6°C and 79.9 and 83%, respectively. The slight differences observed in temperature and relative humidity were not statistically different, thus we can safely conclude that the observed differences in sweat loss were due to the change in the level of protein intake. A summary of nitrogen balance at the respective levels of protein intake (Table 4) illustrates the effects of ignoring sweat nitrogen losses. Nitrogen retention was overestimated without the inclusion of sweat nitrogen.

TABLE 4

Mean* Urinary, Faecal and Sweat Nitrogen Losses and Nitrogen Balance at Four Levels of Dietary Protein Intake.

Level	NI	UN	FN	SN	NB	
					I-U-F	I-U-F-S
I	48.5 ± 5.7	41.4 ± 4.7	15.3 ± 2.77	5.45 ± 0.79	-9.7	$-15.2^{\$}$
II	66.0 ± 7.5	47.0 ± 3.8	16.6 ± 3.68	6.36 ± 0.70	$+1.0$	$-5.4^{\$}$
III	84.5 ± 9.8	53.6 ± 5.7	16.5 ± 3.83	6.65 ± 0.73	$+12.8$	$+6.2^{\$}$
IV	$101.7 \pm 12.5^{+}$	$64.9 \pm 4.5^{+}$	$16.1 \pm 3.82^{\#}$	$7.10 \pm 0.87^{+}$	$+19.2^{+}$	$+12.1^{\$}$

NI = Nitrogen intake; UN = Urinary nitrogen; FN = Faecal nitrogen; SN = Sweat nitrogen; NB = Nitrogen balance; I-U-F = Intake - Urinary - Faecal nitrogen; I-U-F-S = Intake - Urinary - Faecal - Sweat nitrogen.

* Mean values expressed as mean ± SD of nine subjects.

\+ Mean values significantly different from the other values in the column (2-way ANOVA) at $p < 0.05$.

\# Mean values not significantly different from the other values in the column (2-way ANOVA) at $p < 0.05$.

$ Values of nitrogen balances are significantly different (paired t-test at $p < 0.05$).

In experiment 2, sweat nitrogen was found to be 9.74±0.44 mg N/kg/day. Table 5 shows the mean sweat loss collected twice during the experiment between days 18-20 (first dietary interval) and 38-40 (second dietary interval). Their sweat loss was expressed as mg N/kg/day as well as per total body surface area (mg N/m^2/day). The subjects had a fairly constant sweat loss which did not change with time. Atmospheric temperature was also found to be fairly constant throughout the period of the experiment. Mean temperature of the two periods of sweat collection was recorded at 24.5°C.

TABLE 5

Sweat Nitrogen Losses of Six Young Women an 40-Day Continuous Feeding on 0.6 g Protein/kg/day

Nitrogen Loss in Sweat	Days 16-20		Days 38-40	
	Mean	SD	Mean	SD
mg N/day	546.90	83.87	547.60	88.04+
mg N/m^2/day	333.10	25.13	343.40	27.04+
mg N/kg/day	9.74	0.42	9.75	0.35+

* All values are expressed as mean ± SD of six subjects.
+ No significant defference between days of collection (paired t-test at $p < 0.05$).

DISCUSSION

Mitchell and Hamilton (15) were the first to propose that sweat nitrogen loss be included in calculations of human protein requirement because they could not observe any compensatory decrease in urinary nitrogen when sweat nitrogen increased during a hot and humid period. Thus a 13-14% increase in protein requirement was proposed for hot humid tropical areas based on subsequent studies (4). This became a controversial issue as many other workers were able to observe a compensatory reduction of urinary nitrogen with fully acclimatized men during sweating periods (5,7,8,9). It was also observed that the increased sweat nitrogen loss did not result in increased total nitrogen losses after a period of adaptation (9), thus the protein requirements of people living in the tropical areas are possibly not higher than those living in the temperate zones. Therefore, it is very essential that the nitrogen loss through sweat should be determined in nitrogen balance studies.

In our study, the amount of daily nitrogen loss through the skin by the subjects averaged 286, 328, 350 and 371 mg N/kg/day for the protein intakes of 0.3, 0.4, 0.5 and 0.6 g/kg/day, respectively. On continuous feeding of the estimated protein allowance of 0.6 g/kg/day, sweat nitrogen loss ranged between 483 to 700 mg N/kg/day. These values were much lower than expected from results of earlier workers who studied sweat loss in hot and humid conditions. Consolazio et al. (4) reported a value of 2.63 g/day while Mitchell and Hamilton (15) reported 764 mg $N/kg/day/m^2$. The explanation for our lower values is that our subjects were comfortable and fully acclimatized. They did not do any hard physical work and the temperature was not extreme (about 25°C), thus they were not under any stress. Our studies were conducted between the months of April and July, when environmental temperatures are usually not severe in the southern part of Nigeria (where the studies were conducted).

Lack of acclimatization may have been responsible for the higher sweat nitrogen values found in Consolazio and Mitchell and Hamilton's studies, since they were carried out in temporary, artificiallly hot environments in temperate climates. Thus it is not applicable to persons living in tropical countries. Nevertheless, our values are much higher than values obtained by Calloway et al. (3) for subjects in the temperate climates. They observed a mean value of 112 mg/day as obligatory sweat nitrogen loss and 149 mg N/kg/day for a protein intake of 75g.

Thus the 1985 FAO/WHO/UNU expert Committee (2) set up the allowance for sweat nitrogen loss in the calculation of protein requirement estimation based on the Calloway estimates. However, our range of values compare adequately with results of investigators who worked on highly acclimatized subjects in tropical areas. Darke (16) reported a mean 24 hour sweat loss to be 254 mg of nitrogen in eight African males engaged in sedentary activities. Freyberg and Grant (17) found average daily nitrogen loss of 372 mg. Huang et al. (9) observed a range of 650-750 mg/day while Weiner et al. (18) obtained similar figures for acclimatized Tanzanian men under hot conditions. Huang et al. (9) further observed a seasonal change in the sweat nitrogen loss. Higher skin nitrogen losses were observed during high environmental temperatures but then there was a compensatory significant reduction in urinary nitrogen loss. Thus increased skin nitrogen loss in sweating does not necessarily increase total nitrogen losses.

Data from this study reflected similar observation by Cuthbertson and Guthrie (19) that increasing the nitrogen content of the diet caused an increase in the skin excretion of nitrogen. Dermal nitrogen loss in our study increased with dietary protein significantly ($p < 0.05$) and dermal nitrogen was well related to the blood urea nitrogen. Similar results were observed by Calloway et al. (3).

Nitrogen balances calculated without regard to losses in sweat were overestimated by about 8.0%. This resulted in an underestimation of protein requirements. It is imperative that the sweat loss should be estimated at different levels of dietary protein for effective calculation of protein requirements. Also the 1985 FAO/WHO/UNU allowance for sweat is an underestimation under conditions prevailing in tropical areas.

ACKNOWLEDGEMENTS

The authors are grateful to the United Nations University, University of Ibadan and Nigerian Institute of Social and Economic Research (NISER) for making grants available for this work. We are grateful to Professor D.H. Calloway and Professor N. Scrimshaw for their valuable suggestions, advice and critical evaluation of the manuscript. We would like to acknowledge the co-operation of the subjects.

REFERENCES

1. Irwin MI, Heysted DM. A conspectus of research of protein requirement of man. J. Nutr. 1971; 101:385-430.

2. Energy and protein requirements. Report of joint FAO/WHO/UNU Adhoc Expert Committee. WHO Tech. Ser. 1985:No. 724.

3. Calloway DH, Odell ACF, Margen S. Sweat and miscellaneous nitrogen losses in human balance studies. J. Nutr. 1971; 101:775-86.

4. Consolazio DF, Nelson RA, Matoosh CO, Harding RA, Canham JE. Nitrogen excretion in sweat and its relation to nitrogen balance requirements. J. Nutr. 1963; 79:399-404.

5. Ashworth A, Harrower ADB. Protein requirements in tropical countries: Nitrogen balance. Br. J. Nutr. 1967; 21:833-43

6. Howat PM, Korslund MK, Abernathy RP, Ritchey SJ. Sweat nitrogen losses by and nitrogen balance of preadolescent girls consuming three levels of dietary protein. Am. J. Clin. Nutr. 1978; 28:879-82.

7. Yamada T. Could an increased output of nitrogen in sweat result in an increase in protein requirement in muscular exercise? IX International Congress of Nutrition, Mexico 1972.

8. Huang PC, Chong HC, Rand WM. Obligatory urinary and faecal nitrogen losses in young Chinese men. J. Nutr. 1972; 10:1605-14.

9. Huang PC, Loc HoW. Protein requirements of men in a hot climate: decreased urinary nitrogen losses concomittant with increased sweat nitrogen losses during exposure to high environmental temperature. Am. J. Clin. Nitr. 1975; 28:494-501.

10. Atinmo T, Mbofung CMF, Egun G, Osotimehin B. Nitrogen balance study in young Nigerian adult males using four levels of protein intake. Br. J. Nutr. ; 60:451-58.

11. Spence NP, Abernathy RP, Ritchey SJ. Excretion of nitrogen in sweat by preadolescent girls consuming low protein diets. Am. J. Clin. Nutr. 1972; 25:275-78.

12. Sirbu ER, Margen S, Calloway DH. Effect of reduced

protein intakes on nitrogen loss from the human integument. Am. J. Clin. Nutr. 1967; 20:1158-65.

13. AOAC (Association of Official Agricultural Chemists) "Official Methods of Analysis" 1970; 10th ed. AOAC, Washington DC.

14. March WH, Fingerhot B, Miller H. Automated and manual direct methods for determination of blood urea. J. Clin. Chem. 1965; 11 (6):624-27.

15. Mitchell HH, Hamilton TS. The excretion under controlled environmental conditions of nitrogen and minerals in human subjects, with particular reference to calcium and iron. J. Biol. Chem. 1949; 178:345-61.

16. Darke SJ. The cutaneous loss of nitrogen components in African adults. Br. J. Nutr. 1960; 14:115-19.

17. Freyberg RH, Grant RL. Loss of minerals through the skin of normal humans when sweat is avoided. J. Clin. Invest. 1937; 16:729-31.

18. Weiner JS, Wilson JOC, El-Neil H, Wheeler EF. The effect of work on dietary intake on sweat nitrogen losses in a hot climate. Br. J. Nutr. 1972; 27:543-52.

19. Cuthbertson DP, Guthrie WSW. The effect of variations in protein and salt intake on the nitrogen and chloride contents of sweat. Biochem. J. 1934; 28:1444-53.

Accepted for publication January 22,1992.