Effect of Protein Depletion on Urinary Nitrogen Excretion in Undernourished Subjects

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ABSTRACT The extent of labile protein stores and their response to protein feeding was studied in undernourished adult subjects. Urinary nitrogen excretion following 3 levels of protein intake (62, 82 and 96 g/day), each protein period alternated with a protein-free diet, was studied in 4 apparently healthy but undernourished young men. Urinary nitrogen with a protein-free diet decreased to a nearly steady value within 2 to 3 days. The initial decrease was small, however, indicating poor labile protein stores in these subjects. With diets containing protein considerable nitrogen was retained. The retained nitrogen, however, was not excreted when the subjects were changed over from protein-containing diets to protein-free diets. Endogenous urinary nitrogen excretion in these subjects was not different from that of normal subjects.

There have been several studies of the effect of variation of dietary protein intake on the urinary nitrogen excretion (1–3). These studies indicate that there are apparently some components in the body which are comparatively more sensitive to changes in protein intake than others. One of these, body protein, which is particularly sensitive to changes in protein intake, has been termed the labile protein store. The presence of labile protein stores in different species of animals has been inferred from urinary nitrogen excretion immediately following consumption of a protein-free diet (1, 2, 4). Whenever the level of protein intake is altered, there is often a time lag in the adjustment of the body to a new level of protein, which is reflected in a corresponding variation in the excretion of nitrogen. Several investigators have observed (5–11) that in human subjects, a protein-free diet following a normal protein intake causes urinary nitrogen to decrease rapidly at first and then slowly to reach a fairly steady level which has been termed the endogenous level. Extra nitrogen lost in the first few days over and above the endogenous level has been considered to correspond to the labile protein store. According to Martin and Robison (7), this labile store represents about 3% of total body proteins, and under the most favorable circumstances does not exceed 5% (3).

The labile protein stores are drawn upon during periods of stress (12). Although the actual physiological significance of labile protein stores is not fully understood, it is agreed that adequate stores of labile proteins are consistent with good protein nutritional status. In population groups habitually subsisting on marginal intakes of protein, labile protein stores may be expected to be low. In any consideration of protein requirement, in addition to the protein requirement for maintaining N balance protein required to maintain labile protein stores merits attention.

The present paper describes studies of nitrogen balance in apparently healthy but undernourished subjects eating protein-free diets, and the effects of fluctuation in the levels of protein intake on the pattern of urinary nitrogen excretion.

MATERIALS AND METHODS

Subjects. Four apparently healthy young men belonging to the low income group were the subjects of this study. They were kept in a metabolic laboratory during the entire period of the experiment. The basal metabolic rates of these subjects were determined initially. Details of the subjects are shown in table 1.

Diet: The subjects were given a protein-free diet alternating with a high pro-

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TABLE 1
Description of the subjects

Subject	Age	Weight	Height	Surface area	Basal energy
	years	*kg*	*cm*	*cm²*	*kcal/day*
JB	25	44.8	161.4	1.43	1292
MS	26	45.5	162.6	1.44	1076
JS	31	48.4	166.0	1.50	1272
NKS	28	45.4	163.1	1.46	1250

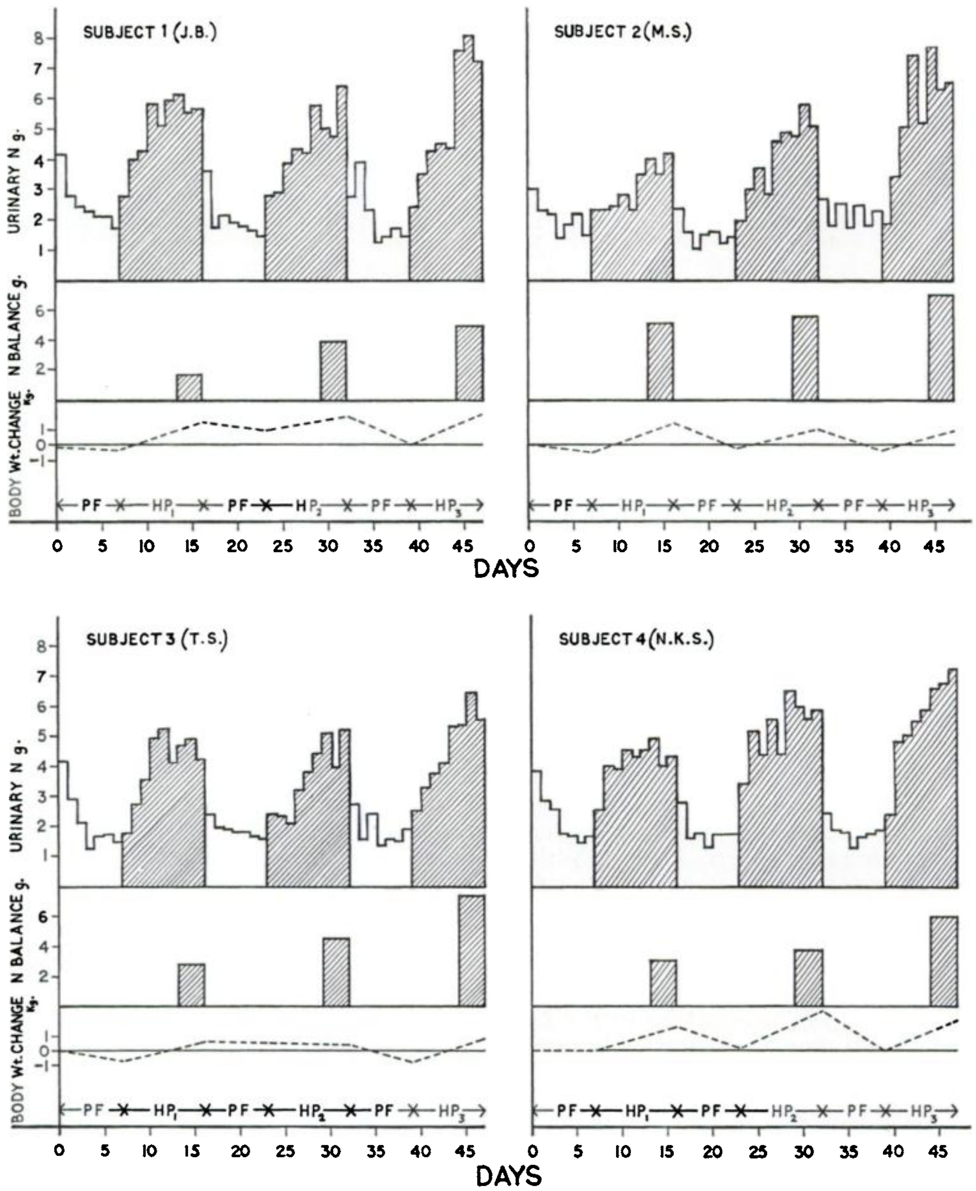

Fig. 1 Nitrogen metabolism of men eating protein-free (PF) and high protein diets (HP_1, HP_2 and HP_3).

tein diet. Each subject was studied for 3 separate periods with the protein-free diet, with each period followed by a high protein diet. Daily protein intake during the 3 protein periods was 62, 82 and 96 g, respectively. The duration of each protein-free dietary period was 7 days and that of the high protein period was 9 days. The sequence of feeding the different diets is shown in figure 1. The protein-free diet included sago, cornstarch and vegetables low in nitrogen and did not contain any protein food. In formulating the practical protein-free diet, however, small quantities of nitrogen from sago and vegetables could not be avoided entirely. Protein in the high protein diets was derived mainly from rice, wheat, red gram dhal (*Cajanus cajan*) and milk. The composition of the diets is shown in table 2.

Urine was collected daily on glacial acetic acid and toluene during the entire period of the study. Feces were collected during the last 3 days of each period. Body weights were taken at the beginning and at the end of each dietary period. Total nitrogen was estimated in urine and feces by the macro-Kjeldahl method.

RESULTS

Average daily urinary nitrogen output, nitrogen balance with the high protein diets and body weight changes during the different dietary periods are shown in figure 1. Body weight changes during the different periods were 1 to 2 kg. Body weight lost during the protein-free regimen was largely made up during subsequent protein feeding.

Urinary excretion of nitrogen decreased rapidly in all the subjects to about 2 g within 2 to 3 days of the protein-free regimen and remained steady or decreased rather slowly thereafter. The magnitude of the initial decrease in urinary nitrogen excretion with the protein-free regimen, however, was quite small in these subjects, unlike the sharp decrease in normal subjects eating protein-free diets that was reported by Martin and Robison (7). Moreover, the 2 distinct phases of urinary nitrogen excretion reported by other workers were not strictly in existence in all the subjects. This indicated that the significant initial response to the protein-free diet reported in well-fed subjects was absent or minimal in our subjects. When our subjects were fed a high protein diet following the protein-free diet, urinary nitrogen excretion increased rapidly and tended to reach a plateau. Even after 9 days of protein feeding the subjects had not attained N equilibrium.

Average daily urinary nitrogen excretion during the last 3 days of the protein-free diet is shown in table 3. Following the usual convention, these values were taken to represent endogenous excretion

TABLE 2

Composition of experimental diets

	Protein-free diet	Protein diets 1	Protein diets 2	Protein diets 3
	g	*g*	*g*	*g*
Sago	255	—	—	—
Rice	—	300	400	450
Wheat flour	—	100	133	170
Cornstarch	210	—	—	—
Red gram dhal	—	50	66	100
Skim milk powder	—	20	27	40
Sugar	90	60	52	28
Oil	90	40	10	20
Calabash cucumber	240	—	—	—
Ridge gourd	250	—	—	—
French beans	—	200	250	275
Potato	—	200	300	—
Cauliflower	—	100	100	100
Onions	—	50	50	25
Protein, g	3.4	62.4	81.6	95.7
Kilocalories	3007	3004	3006	3022

TABLE 3

Nitrogen output and body nitrogen loss of men eating a protein-free diet

Subject	Avg daily N output during last 3 days of protein-free diet period						Body nitrogen loss [1] during protein depletion period		
	Period 1		Period 2		Period 3				
	Urinary	Fecal	Urinary	Fecal	Urinary	Fecal	Period 1	Period 2	Period 3
	g	g	g	g	g	g	g	g	g
JB	1.94	0.91	1.61	1.39	1.57	1.36	3.82	2.86	4.02
MS	1.81	0.85	1.39	0.99	2.18	1.02	2.73	0.81	0.55
JS	1.62	0.89	1.66	1.03	1.66	1.37	3.83	1.42	1.47
NKS	1.57	1.00	1.70	0.91	1.75	1.13	4.60	0.78	0.34

[1] Urinary nitrogen excreted over and above the endogenous minimum.

of nitrogen. Endogenous urinary nitrogen values with the protein-free diet were almost the same in the 3 protein-free periods irrespective of the protein intake preceding each period and ranged from 1.5 to 2.0 g/day. The endogenous urinary N output per basal kilocalorie ranged from 1.30 to 1.65 mg, the average for the 4 subjects being 1.4 mg/basal kcal. This value is not different from values for normal subjects reported by other workers (10, 13–15). Fecal N excretion with the protein-free diet ranged from 0.89 to 1.37, the average value being 1.07 g/day. This value is not very different from the values reported by Murlin et al. (13), and Mueller and Cox (11) in two of his subjects, but it is higher than values reported by Bricker and Smith (14) and Hawley et al. (15).

Extra urinary nitrogen lost over and above the endogenous minimum during the protein depletion period after the subjects were transferred to the protein-free diet is also shown in table 3. Body nitrogen loss during this period ranged from 0.34 to 4.6 g which was less than 1% of body protein in the 4 subjects. This loss was highest in the first protein-free diet period and decreased in the subsequent 2 protein-free diet periods in three of the four subjects. In subject JB nitrogen loss did not differ greatly in the 3 protein-free diet periods. Short periods of high protein feeding prior to use of the protein-free diet did not increase the loss of body proteins observed with protein-free diet.

The average values for daily nitrogen retention during the last 3 days of the 3 high protein regimens are shown in figure 1. Total nitrogen retained during the entire period of 9 days would be much larger. Nitrogen retained even during the last 3 days of the diet was considerable, the higher the protein level in the diet the more nitrogen retained. These results suggested that nitrogen retained during the high protein regimen was substantially higher than nitrogen lost during the preceding protein-free dietary period.

DISCUSSION

The initial rapid urinary nitrogen loss following the protein-free diet has been considered to represent loss of labile protein stores of the body. The decrease in urinary nitrogen excretion following a protein-free diet in well-nourished individuals consists of 2 phases, one rapid phase when the urinary nitrogen excretion decreases to a low value within a few days and the other a slow phase when the nitrogen excretion decreases rather slowly until it reaches a steady state. This pattern has been observed both in human subjects and experimental animals (1-4). Nitrogen loss in the first phase is considered to represent loss of labile protein stores which may be 3 to 5% of body proteins. Nitrogen loss in the subsequent slow phase may represent loss of more stable body proteins. In the present study which concerned relatively undernourished subjects, however, the pattern of urinary nitrogen loss with a protein-free diet appeared to correspond mainly with the slow phase, the rapid phase being absent or minimal. This indicated that highly labile protein stores such as those observed in normal persons were minimal or absent in these subjects. This conclusion is supported further by the fact that the calculated body nitrogen loss in our subjects during the protein-free regimen was less than 1.0% of body nitrogen compared with a loss of 3 to 5% of body nitrogen reported for well-nourished subjects. Relative tissue depletion in our subjects is also supported by the tendency of the subjects to retain large quantities of nitrogen when they were maintained with a high protein diet following a protein-free diet. The subjects of this study normally subsisted on marginal protein intakes and it may be argued that they could not have built up adequate labile protein stores. It appears from this study that the pattern of body protein loss with a protein-free diet depends upon the state of protein stores in the body. The response to a protein-free diet can be expected to be poor, as in our subjects, if only less labile proteins are present in the body since this component of protein could be expected to resist depletion. The significance of the poor response of our subjects to a protein-free diet in terms of their proteins stores needs further study.

Loss of urinary nitrogen during the 3 protein-free periods apparently was not influenced by the preceding short periods of high protein feeding which resulted in a large positive nitrogen balance. Nitrogen retained with high protein feeding was obviously not excreted when these subjects were transferred to a protein-free diet, a deviation from normal response. This can be interpreted to mean that these subjects had extensively depleted protein stores and that nitrogen retained by them was presumably repleting preferentially less dispensable body protein stores and not highly labile protein stores. Perhaps a prolonged period of high protein feeding to these subjects to the point where their body protein stores were completely filled would be necessary before they could respond to a protein-free diet as well as well-nourished subjects.

One of the points of practical significance in the present study concerns the protein requirement of undernourished subjects. Since endogenous nitrogen excretion in our subjects was not different from that reported for normal subjects, the minimal protein requirement of these subjects necessary to take care of the obligatory loss is apparently not different from that of normal well-nourished subjects. Since these subjects usually have very poor stores of reserve proteins, a protein allowance to cover only the minimal needs would never provide an opportunity for them to replete their body protein stores fully. Therefore, the basis for computation of the protein requirement in such subjects would obviously differ from that for normal subjects. Although the significance of labile protein stores in normal subjects is not understood clearly, its beneficial effect during times of stress may be important.

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