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Original Communications

Nitrogen Retention in Adult Man: A Possible Factor in Protein Requirements[1, 2, 3]

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MOST STUDIES of the protein requirement for adult man have been concerned with the measurement of nitrogen (N) retention on minimal-protein intakes (1). However, the recommended protein allowance of 1 g/kg body wt, as suggested by the National Research Council (2), takes into account the possibility of protein needs to fill and maintain protein reserves. According to Holt et al. (3) surfeit-protein feeding of adult subjects is unwarranted since *a*) there is doubtful evidence for the storage of surfeit protein in the adult and *b*) Holt et al. (3) found no evidence for the utilization of protein stores in young growing rats. With regard to the latter argument, Fisher et al. (4) have presented evidence for the utilization of excess N stores under stress of disease and amino acid imbalance.

Cuthbertson et al. (5) and Holmes et al. (6) have suggested the possibility of protein storage following high-protein intake in adult human subjects. An important difference between the studies of Holmes and associates and those of Cuthbertson and associates was the observation by the former workers that on high-protein intakes considerable N retention occurred without concomitant increases in body weight. The latter workers felt that they could account for most of the retained N in terms of increases in body weight.

Fisher et al. (7) have also encountered positive N retention of a magnitude greater than expected in young men on relatively high-protein intakes (18–20 g N/day). The present studies were undertaken to expand the investigations of the effects of high dietary-N intakes from good quality protein on N retention, certain blood and urine constituents, and on body composition.

EXPERIMENTAL

Three studies, of 10, 7, and 4 weeks' duration, were carried out with six, four, and four subjects, respectively. The subjects were 20- to 22-year-old male college students, with body weights ranging from 61.0 to 79.1 kg and heights between 168–188 cm some of whom had

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participated in earlier studies (7). Different levels of dietary N from good quality proteins were supplied for periods of 1–4 weeks. Most of the results are calculated and presented in terms of average weekly observations.

Urine was collected and analyzed on a 24-hr basis for 4 days out of every week; fecal collections were pooled for 7-day periods during each dietary regimen. The feces were collected in 1-gal paint cans with sealed lids, and dried at 40 C in a forced-draft oven before grinding and sampling for N analyses.

The subjects were weighed daily before breakfast. Every effort was made to maintain a similar daily rate of activity while the subjects went about their routine on a nonconfined basis. All meals were taken in the Nutrition Department's experimental dining room and corrections were made for N losses due to adhesion of food on the dishes based on analyses of 24-hr washing residues. Blood was taken for analysis before breakfast on the 6th day of each week. Nitrogen losses from the skin were not measured in this study (see first paragraph of RESULTS).

Most of the analytical methods employed have been described previously (7). Determinations were made of urinary and fecal N, urinary creatinine, plasma protein, urea N and amino acid N, and serum-free amino acids. Methods not previously described include the determination of plasma proteins on the Technicon AutoAnalyzer according to the procedure of Failing et al. (8) and the determination of serum-free amino acids on a Phoenix amino acid analyzer by the method of Piez and Morris (9). In the second experiment total-body K was determined using a whole-body counter which measured the ^{40}K content of the subjects at the end of the 1st week (on the 15 g N/day diet) and again at the end of the 7-week experimental period (following ingestion of 26 and 34 g N/day, respectively, for 3-week periods). In the third experiment body K measurements were similarly determined at the start and at the end of the 4-week high-protein intake period. The equipment, procedures, and standardization techniques (by the meter-arc calibrating method) were essentially the same as those used at the Argonne National Laboratory, as described by Marinelli et al. (10). In the second experiment each subject was counted for a 30-min period and in the third experiment for 45 min; standard errors were calculated from the counts during this time interval. The high standard errors at the end of *experiment 2* were due to the application of a correction factor as a result of a modification in the distance between subject and the counting crystal which was undertaken in the chamber following the initial measurements.

The basic diet, which supplied approximately 15 g N/subject per day, included the following items: one egg, 28 g corn flakes, 500 g milk, 113 g orange juice, one American cheese sandwich (56 g American cheese), one bologna sandwich (60 g bologna), three additional slices white bread (total of seven slices bread providing 14 g protein), 56 g butter, 14 g jelly, 100 g peaches, 100 g lean, raw meat, 20 g dried potato mix, 80 g frozen peas, 30 g lettuce, one 60-g slice of ice cream, and 168 g tomato juice. The higher-protein diets were prepared by adding an additional egg and additional quantities of lean meat and of dry skim milk powder. The low-protein diet (used only during the final 2 weeks of the first experiment) was prepared by reducing the quantity of meat and milk and by omitting the egg from the basic diet listed above. The mean caloric intake of the subjects was 2,850 on the 9-g N diet, 2,723 on the 15-g N diet, 3,233 on the 26-g N diet, 3,199 on the 27-g N diet, and 4,088 on the 34-g N diet. While a part of the variation in caloric intake was probably due to differences in activity among subjects, the major differences were related to the necessity of increasing the caloric content of the diet in order to increase its protein content. No attempt was made to compensate for these increases in caloric intake by greater activity.

RESULTS

The results of the first experiment (Table 1) indicate some N retention on the 15-g N/day intake even when allowance is made for N losses from the skin, which were not measured (0.5–1.0 g N/day (11)). When dietary N was increased from approximately 15 to 25 g/day, strong positive N retention occurred throughout the 3-week period on this level of N intake. On return to the lower N intake (15 g/day), retention decreased to the level observed earlier. As expected, the subjects were still in N equilibrium on a daily intake of 9 g

TABLE I

Nitrogen Balance and Plasma- and Urinary-N Constituents of Six Male College Students Receiving Different Amounts of Dietary N from Good Quality Protein

Experimental Period	Dietary N, g/day[a]	Daily N Balance, g	Urinary Creatinine, g/day	Plasma		Body-Weight Change, kg
Week				Urea N, mg/100 ml	Amino acid N, mg/100 ml	
1	14.82	1.90 ± 0.65[b]	1.67 ± 0.07	14.7 ± 0.80	5.43 ± 0.05	−0.04
2	14.82	2.67 ± 0.46	1.82 ± 0.06	13.8 ± 0.56	5.48 ± 0.08	−0.15
3	14.82	2.01 ± 0.24	1.64 ± 0.08	11.4 ± 0.45	5.33 ± 0.17	−0.26
4	24.88	7.40 ± 0.58	1.57 ± 0.07	15.7 ± 1.69	5.52 ± 0.11	+0.75
5	24.88	5.40 ± 0.41	1.88 ± 0.12	14.9 ± 0.71	5.67 ± 0.20	0
6	24.88	7.72 ± 1.12	1.70 ± 0.02	13.8 ± 0.71	6.17 ± 0.25	−0.41
7[c]	15.34	2.39 ± 1.05	1.50 ± 0.03	12.5 ± 0.89	5.45 ± 0.10	+0.82[c]
8	15.34	2.54 ± 0.28	1.71 ± 0.05	13.0 ± 0.67	5.76 ± 0.10	−1.21
9	8.85	0.83 ± 0.26	1.69 ± 0.04	10.7 ± 0.27	4.37 ± 0.08	+0.29
10	8.85	2.21 ± 0.13	1.60 ± 0.05	10.0 ± 0.32	4.37 ± 0.06	+0.07

[a] See text for diet composition.

[b] Standard errors were calculated from the mean values for each of the six students; the value for each student represents the mean of four 24-hr collections in the case of urinary constituents and N balance and a single weekly value for the plasma analyses.

[c] During the 7th week only three 24-hr collections were made because of Thanksgiving when the subjects were permitted a 3-day respite. The relatively large increase in body weight during this period was apparently due to this holiday weekend.

N. The body-weight changes, particularly during the first 6 weeks on the high-N intake, were negligible and did not account for the amount of N retained. Of the other measurements made during this experiment, the only significant changes were the lowered plasma–urea N and amino acid-N levels on the 9-g N/day intake in comparison with the higher-N intakes.

Experiment 2 was essentially a more elaborate repetition of *experiment 1* (the four subjects used had all participated in *experiment 1*), which included a still higher level of dietary N (34 g). In this trial an attempt was made to ascertain if the amount of N retained could be accounted for in terms of changes in body protein (as calculated from body-K measurements).

The results of *experiment 2* (Table II) corroborate the observations made in *experiment 1*. The subjects retained considerable quantities of dietary N throughout the 7-week experimental period. During the final week there was a distinct drop in N retention, suggesting a quantitative limitation to body-N stores. In fact, one of the four subjects was essentially in N equilibrium on the final day of the study.

There was a significant increase in the urinary creatinine excretion with increasing intake of dietary N (Table II). No change was noted in plasma-protein level throughout the 7-week study, but there was a highly significant increase in the level of plasma urea with increasing N intake. Little change was observed in the plasma amino acid-N level.

The comparison of serum-free amino acids (Table III) before and after the ingestion of the high-N diets (15 vs. 26 and 34 g N/day) also showed only a small increase

TABLE II

Nitrogen Balance and Plasma- and Urinary-N Constituents of Four Male College Students Receiving Relatively High Amounts of Dietary N from Good Quality Protein

Experimental Period	Dietary N, g/day[a]	Daily N Balance, g	Urinary Creatinine, g/day	Plasma		
Week				Protein, g/100 ml	Urea N, mg/100 ml	Amino acid N, mg/100 ml
1	14.67	3.82 ± 0.54[b]	1.68 ± 0.04	7.09 ± 0.15	12.18 ± 0.78	5.14 ± 0.15
2	26.33	7.90 ± 0.48	1.68 ± 0.04	7.27 ± 0.08	13.04 ± 0.65	5.88 ± 0.20
3	26.33	5.87 ± 1.27	1.80 ± 0.06	6.93 ± 0.07	15.54 ± 0.62	5.38 ± 0.09
4	26.33	4.98 ± 0.64	1.79 ± 0.08	7.47 ± 0.07	15.96 ± 0.25	5.92 ± 0.19
5	33.81	5.25 ± 0.87	1.97 ± 0.04	7.42 ± 0.06	16.90 ± 0.22	5.68 ± 0.15
6	33.81	4.73 ± 0.91	1.94 ± 0.04	6.94 ± 0.15	19.06 ± 1.64	5.88 ± 0.07
7	33.81	3.14 ± 0.69	2.04 ± 0.04	7.05 ± 0.17	19.96 ± 0.55	5.84 ± 0.09

[a] See text for diet composition.

[b] Standard errors were calculated from the mean values for each of the four students; the value for each student represents the mean of four 24-hr collections in the case of urinary constituents and a single weekly value for the plasma analyses.

in total amino acids. However, with the exception of threonine all the essential amino acid levels increased while the changes in nonessential amino acids did not follow a clear pattern. The increase in the ratio of essential to nonessential amino acids upon the prolonged high-protein intake approached significance ($P < 0.1$).

When the expected body-weight changes were calculated from the N retention values (N balance, Table III) poor agreement with the observed weight changes was noted particularly for 3 out of 4 subjects (Table IV). Retained N could also not be accounted for in terms of body composition changes measured as body-K concentration (Table IV).

Experiment 3 (Tables V and VI) further confirmed the results of the earlier studies. In this experiment different subjects were used. Nitrogen retention was substantial and of similar magnitude as observed during *experiment 2* on a similar N intake. There was a significant increase in urinary creatinine excretion between the first and the last week on the high-protein diet (Table V).

There was again poor agreement between expected and observed body-weight changes (Table VI) and the small, nonsignificant increase in body K also did not account for the considerable amount of retained N.

DISCUSSION

This investigation corroborates the observation of Holmes et al. (6) that healthy adult subjects can retain relatively large amounts of N from good quality, high-protein diets without concomitant changes in body weight. The absence of a significant change in body-K content suggests that a major part of the retained N must be present as nonprotein N. This observation supports the findings of Shapiro and Fisher (12) and of Basak (13). The former workers showed that partial repletion of body-N stores could be attained by nonessential-amino acid N in adult cocks previously depleted to the endogenous level of N excretion. Basak (13) showed that the N losses of human subjects on a protein-free diet were primarily nonprotein in origin and represented about 6% of the

total-body N of human adults on a normal diet. This quantity is similar to the estimated magnitude of N stores in the adult cock (6.9% of body N) as measured by the depletion technique of Wessels and Fisher (14). In view of the apparent nonprotein nature of the stored N it seems appropriate to recommend the use of the term "N stores" to replace "protein reserves."

It was interesting to note (Table III) that little of the additionally retained N was present in the form of free serum amino acids. The meaning of the observed increase in the essential-to-nonessential amino acid ratio is not clear, but is similar to the increase reported by Swendseid et al. (15) with increased protein intake. The essential-to-nonessential amino acid ratio of the dietary proteins actually decreased slightly between the 15 g N/day diet and the diet supplying the higher quantity of protein.

TABLE III

Serum-Free Amino Acid Concentration at the Start and End of a Period of High-Protein Intake

Amino acid	Serum Level, μmoles/100 ml	
	After 1 week	At end of experiment
Essential		
Lysine	15.1 ± 3.3	20.6 ± 0.6
Threonine	12.5 ± 4.3	11.5 ± 1.0
Valine	18.7 ± 2.9	27.8 ± 1.6
Leucine	9.4 ± 1.1	13.3 ± 1.4
Isoleucine	7.0 ± 1.3	8.3 ± 1.0
Methionine	2.1 ± 0.3	2.5 ± 0.2
Phenylalanine	5.1 ± 0.9	6.4 ± 0.6
Total	69.8 ± 10.7	90.2 ± 6.5
Nonessential		
Aspartic acid	1.4 ± 0.1	1.7 ± 0.2
Glutamic acid	9.3 ± 2.2	11.6 ± 0.6
Alanine	26.4 ± 2.1	26.3 ± 3.1
Glycine	15.3 ± 2.1	14.5 ± 1.0
Serine	9.0 ± 1.4	9.9 ± 0.6
Proline	19.0 ± 5.2	24.9 ± 0.5
Half-cystine	11.8 ± 1.9	11.2 ± 0.3
Tryptophan	4.9 ± 1.2	6.6 ± 1.7
Histidine	9.8 ± 1.8	11.9 ± 0.7
Arginine	8.6 ± 2.0	10.2 ± 1.1
Total	117.8 ± 18.5	128.5 ± 7.5
Essential-to-nonessential ratio	0.59 ± 0.01	0.70 ± 0.01

TABLE IV

Observed and Expected Body-Weight Change and Body-Potassium Measurements

Subject	Body-Weight Change		Body K	
	Observed, kg	Expected, kg[a]	Start, mEq	End, mEq
1	+3.6	+4.1	3,931 ± 123[b]	3,795 ± 300
2	+1.6	+5.0	4,167 ± 128	4,295 ± 387
3	+1.5	+4.0	3,931 ± 126	3,846 ± 305
4	+4.5	+5.9	3,467 ± 120	3,518 ± 333

[a] The total N retained by each subject was calculated by summating the products of 7 times the average weekly retention for the 7-week experimental period; the value thus obtained was converted to tissue gain using the factor 18.75 (6.25, to convert N to protein; 3, to allow for the water to be retained with the protein) as suggested by Holmes et al. (6).

[b] Mean ± SE of a single measurement of ^{40}K.

One of the intriguing aspects of the relatively high degree of N retention of our subjects concerns their protein intake prior to the start of the present studies. According to diet recalls, all subjects consumed between 100 and 150 g protein daily from foods which are generally regarded as sources of good quality protein. The question arises why, from protein intakes of the magnitude mentioned, the N stores of the subjects were not previously at maximum capacity. It is apparent that many questions, such as the total amount of protein consumed at a given meal, the calorie–protein balance at the dinner meal, which usually provides the largest daily protein quantity, and perhaps others, remain to be answered concerning this aspect of protein metabolism relative to our eating habits.

TABLE V

Nitrogen Balance and Plasma- and Urinary-N Constituents of Four Male College Students on a High-Protein Diet for 4 Weeks

Experimental Period	Dietary N, g/day[a]	Daily N Balance, g	Urinary Creatinine, g/day	Plasma	
Week				Urea N, mg/100 ml	Amino acid N, mg/100 ml
1	27.60	6.53 ± 0.60[b]	1.49 ± 0.05	22.12 ± 2.63	6.00 ± 0.16
2	27.60	5.82 ± 0.60	1.53 ± 0.05	22.40 ± 0.55	5.56 ± 0.19
3	27.60	5.74 ± 0.60	1.65 ± 0.07	23.10 ± 1.45	5.60 ± 0.05
4	27.60	6.38 ± 0.43	1.80 ± 0.09	19.44 ± 1.04	6.10 ± 0.17

[a] See text for diet composition.

[b] Standard errors were calculated from the mean values for each of the four students; the value for each student represents the mean of four 24-hr collections in the case of urinary constituents and a single weekly value for the plasma analyses.

TABLE VI

Observed and Expected Body-Weight Change and Body-Potassium Measurements

Subject	Body-Weight Change		Body K	
	Observed, kg	Expected, kg[a]	Start, mEq	End, mEq
1	+0	+3.4	3,787 ± 102[b]	3,977 ± 108
2	+1.8	+4.0	3,431 ± 102	3,725 ± 108
3	+3.8	+2.7	3,587 ± 102	3,854 ± 108
4	+0.4	+2.8	3,759 ± 102	3,990 ± 108

[a] The total N retained by each subject was calculated by summating the products of 7 times the average weekly retention for the 4-week experimental period; the value thus obtained was converted to tissue gain using the factor 18.75 (6.25, to convert N to protein; 3, to allow for the water to be retained with the protein) as suggested by Holmes et al. (6).

[b] Mean ± SE of a single measurement of ^{40}K.

An even more important question, in the light of these observations, is that of the usefulness, to the individual, of N thus retained from surfeit-protein intake. It should be stated, with the full realization of the subjectivity of the observation, that all subjects remarked as to the conspicuous absence of respiratory ailments during this study which was carried out in winter during a period of relatively high incidence of influenza among the general student population.

If, in the light of earlier observations (4, 16), the usefulness of N stores is established also for adult man, the optimum protein intake for maintenance will need to be reexamined. A step in this direction was recently reported by Wannemacher and McCoy (17) who noted a maintenance protein requirement of 7 mg N/basal calorie for adult dogs with full protein reserves, in contrast to a minimal requirement of only 2 mg N/basal calorie for dogs without such stores.

In an earlier study (18) it was observed that creatinine excretion for both man and rat was related to the dietary-N intake. The increased excretion of urinary creatinine which was observed in some of the present experiments with high levels of N intake could, at least in part, have been the result of an increased creatine ingestion from the larger amount of meat which was used in the high-protein diets. This explanation is suggested by the work of Bleiler and Schedl (19); the earlier studies of Rose and Dimmitt (20) suggest, however, that quantities of creatine considerably greater than those added by the meat (approximately 400 mg/100 g meat) would have been necessary to account for

the increase in creatinine excretion observed in these experiments. An alternative explanation would be an increase in body protein which, however, could not be substantiated by the body-^{40}K measurements.

SUMMARY

Experiments were undertaken to investigate the effects of high dietary-N intakes from good quality protein on N retention and on body composition. Diets supplying from 9 to 34 g N/day were given to 20- to 22-year-old male college students. On dietary-N intakes in excess of 15 g/day, considerable quantities of N were retained over periods of several weeks. The total quantity of N retained could not be accounted for in terms of observed body-weight changes, or body-composition measurements based on whole-body ^{40}K concentrations. Thus, a major portion of the stored N appears to be retained as nonprotein N. The amount of N retained on the high-protein intakes was particularly intriguing in view of the relatively high-protein intake of these subjects prior to their participation in the present diet studies. The full significance of such N stores awaits further investigation.

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