Utilization of Urea Nitrogen in Papua New Guinea Highlanders

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(Received February 22, 1984)

Summary The utilization of urea nitrogen was examined in 10 healthy adult men from a village near Lufa, in the Eastern Highlands Province of Papua New Guinea. The staple diet of these men was sweet potatoes. [15N]urea was used as tracer for urea released into their intestinal tracts and the utilization of the urea-N was estimated from the trend of 15N. The men were orally given [15N]urea at the beginning of the study and then their daily protein intake, serum protein levels, 15N excretion in the feces and urine, 15N retention in the whole body and 15N incorporation into serum protein were examined. Their daily protein intake (32.2 ± 8.6 g/day) was low, but their serum protein level (8.05 ± 0.41 g/100 ml) was within the normal range. 15N retention in the whole body on day 3 was estimated to be 35.4 ± 20.2% of the total amount administered, calculated from the recoveries in the feces (1.64 ± 0.85%) and urine (63.0 ± 20.5%) on days 1–3. The utilization of urea nitrogen in Papua New Guinea highlanders was confirmed from the finding of 15N incorporation into serum proteins on day 3 (0.008 ± 0.005 atom% excess). This incorporation was negatively correlated with the urinary nitrogen excretion and serum protein level. This correlation suggests that Papua New Guinea highlanders with low urinary nitrogen excretion or a low level in serum protein, who are in a poor state of protein nutrition, tend to utilize more urea nitrogen for the synthesis of serum protein.

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Inorganic nitrogen compounds in the diet are available as nitrogen sources in ruminants. In addition, it is known that monogastric animals, including man, can use the nitrogen of inorganic compounds for the synthesis of amino acids. There is evidence that supplementation of a diet with urea results in an improved nitrogen balance in malnourished men and even a substantial increase in growth of malnourished infants. Urea or ammonium salt added to the diet can be used for synthesis of body protein in subjects on a low-protein diet or a diet containing only essential amino acids as a nitrogen source. These findings have been confirmed from studies on the incorporation of $^{15}$N from labeled urea into serum protein. These results suggest that nitrogen of endogenous urea released into the intestinal tract can be utilized.

Many investigators have reported that sweet-potato eaters of Papua New Guinea subsist on a low protein intake, but that they are not malnourished, and also have suggested the possibility that they undergo metabolic adaptation to a low protein intake. Based on the finding that urea can be utilized during protein deficiency, we postulated that an adaptation to a low protein intake by Papua New Guinea highlanders may be in the form of utilization of the nitrogen of endogenous urea. In a pilot study, Tanaka et al. examined the utilization of urea-N in Papua New Guinea highlanders and found that $^{15}$N given orally as urea was incorporated into amino acids. In the present study, we examined the utilization of urea-N by these highlanders on a traditional low-protein diet containing sweet-potatoes as the staple food to determine the characteristics of the utilization of endogenous urea-N, supposing that $[^{15}N]$urea given orally is metabolized in the same way as endogenous urea released into the intestinal tract. We also investigated whether any factors were related to the extent of urea-N utilization in Papua New Guinea highlanders.

**METHODS**

*Subjects.* Studies were carried out in the village of Kalugaluvii near Lufa in the Eastern Highlands of Papua New Guinea. This study was made on 10 of the 18 adult men used in previous studies for a nutritional survey and on nitrogen balance and hematology. Details of the village and its life-style have been reported elsewhere. The subjects showed normal values in urinary tests for protein, sugar, acetone bodies and urobilinogen. Their average body height and weight were 156.5 ± 5.3 cm and 58.2 ± 6.0 kg, respectively, as shown in Table 1.

For examination of utilization of urea-N, urea labeled with $^{15}$N (95.0 or 95.6 atom%) was administered orally in a single dose of 10 mg $^{15}$N per kg body weight at 06:00 h, before breakfast. The subjects then continued their usual life, except that 24-h urine and stool specimens were collected for the first 3 days of the test period.
Table 1. Age and body height and weight of the Papua New Guinea highlanders studied.

| Subject | Name | Agea (year) | Height (cm) | Body weight (kg) |
|---------|------|-------------|-------------|-----------------|
| 1       | IP   | 24          | 155.0       | 55.0            |
| 2       | AL   | 38          | 158.5       | 58.0            |
| 3       | ON   | 20          | 166.0       | 62.1            |
| 4       | NE   | 40          | 159.0       | 59.0            |
| 5       | FA   | 35          | 154.5       | 55.5            |
| 6       | KA   | 39          | 153.0       | 59.0            |
| 7       | MA   | 38          | 156.5       | 49.3            |
| 8       | HA   | 27          | 145.5       | 50.4            |
| 9       | JO   | 29          | 159.7       | 67.5            |
| 10      | AN   | 33          | 157.0       | 66.0            |

Mean ± SD 156.5 ± 5.3 58.2 ± 6.0

a Estimated by a medical assistant working at the Medical Center in Lufa in Papua New Guinea.

Blood was withdrawn just before [15N]urea administration and on days 3, 5 and 7 after its administration.

Analytes. Total serum protein was determined by the biuret reaction (14) and urinary and fecal nitrogen by a semi-micro Kjeldahl method. The 15N contents of fecal nitrogen, urinary nitrogen, ammonia and urea and serum protein and non-protein nitrogen (NPN) were measured. Samples other than the serum protein fraction were prepared by the method of Kumazawa (15) and subjected to optical spectrographic analysis. The 15N content of serum protein was analyzed by mass spectrometry because the isotope concentration was too low for optical spectrographic analysis. For separation of serum NPN and protein, the serum sample was treated as follows: 2.0 ml of serum was mixed with 20 ml of 1% picrate and centrifuged at 3,000 rpm for 20 min. The protein fraction was hydrolyzed with 6N HCl at 120°C for 24 h, and HCl was removed in an evaporator. Then picrate in the supernatant (NPN fraction) and the hydrolysate (protein fraction) was removed with Dowex 2 x 8 resin.

Fractionation of ammonia and urea plus ammonia in the urine for 15N analysis was achieved by the method of Van Slyke-Cullen with a slight modification. The 15N content of urinary urea was calculated from the 15N concentrations in the fractions of ammonia and urea plus ammonia, and the total amount of nitrogen in urinary urea and ammonia.

RESULTS

Table 2 shows the mean protein intakes per day in the test period and the
Table 2. Protein intake and serum protein levels of the Papua New Guinea highlanders studied.

| Subject | Protein intake\(^a\) (g/day) | Serum protein\(^b\) (g/100 ml) |
|---------|-----------------------------|-------------------------------|
| 1       | 38.7                        | 8.01                          |
| 2       | 19.4                        | 7.73                          |
| 3       | 38.3                        | 8.71                          |
| 4       | 35.4                        | 8.37                          |
| 5       | 26.3                        | 8.19                          |
| 6       | 49.5                        | 8.31                          |
| 7       | 27.4                        | 7.29                          |
| 8       | 30.0                        | 7.61                          |
| 9       | 25.7                        | 8.06                          |
| 10      | 31.1                        | 8.18                          |

Mean ± SD  32.2 ± 8.6  8.05 ± 0.41

\(^a\) Values are averages for individuals for 3 days in the study period. \(^b\) Values are averages for three samples from individuals taken on days 0, 3 and 5.

Table 3. \(^15\)N recovery in feces and urine during 3 days and \(^15\)N retention in the whole body on day 3 as percentages of the administered \(^15\)N.

| Administered \(^15\)N\(^a\) | 581.5 ± 60.1\(^b\) |
|-----------------------------|--------------------|
| Feces (for 3 days)\(^c\)   |                    |
| \(^15\)N atom\(^\%\) excess | 0.20 ± 0.13  |
| N excretion (mg/day)        | 1,566 ± 414   |
| Recovered \(^15\)N\(^d\) (mg)| 9.52 ± 4.95   |
| Recovery\(^e\) (\%)         | 1.64 ± 0.85   |
| Urine                       |                    |
| \(^15\)N atom\(^\%\) excess | 4.99 ± 1.39  |
| N excretion (mg)            | 5.060 ± 1,545 |
| Recovered \(^15\)N (mg)     | 268.9 ± 106.3 |
| Recovery (\%)               | 45.8 ± 17.9   |
| Total recovery\(^f\) (\%)   | 63.0 ± 20.5   |

\(^a\) Administered \([\(^15\)N]urea (95.0 or 95.6 atom\(^\%\)) corresponding to 10 mg \(^15\)N per kg body weight as a single dose at the beginning of the study. \(^b\) Mean ± SD for 10 subjects. \(^c\) Pooled as sample for 3 days of the test period. \(^d\) Calculated from \(^15\)N atom\(^\%\) excess and total nitrogen excretion for 3 days based on the mass weight of heavy nitrogen. \(^e\) \(^15\)N recovered during 3 days as a percentage of the \(^15\)N administered. \(^f\) Total value for urinary \(^15\)N recovery in 3 days. \(^g\) Calculated by subtracting the percentages of \(^15\)N recovered in the feces and urine in 3 days from 100.

\(J.\ Nutr.\ Sci.\ Vitaminol.\)
average serum protein concentrations on days 0, 3 and 5 in the 10 individuals studied. The average protein intake was 32.2 ± 8.6 g per day. This result is consistent with reports by others that Papua New Guinea highlanders had a low protein intake (6–9). However, despite their low protein intake they had a good physique with well developed muscles and showed normal hematological parameters, as reported previously (10, 11, 16). The serum protein level of the subjects in the present study ranged from 7.3 to 8.7 g per 100 ml.

The subjects were given [15N]urea at a dose of 10 mg 15N per kg body weight and their urinary and fecal excretions of 15N were then examined. Table 3 shows results on 15N recoveries in the feces and urine during the first 3 days and 15N retention in the whole body on day 3 as percentages of the dose administered. 15N recovery in the urine was 45.8 ± 17.9% on day 1, 12.9 ± 5.6% on day 2 and 3.9 ± 1.6% on day 3, the cumulative percentage over the 3 days being 63.0 ± 20.5%. 15N atom% excess in the feces was only 0.20 ± 0.13%, and about 1.6% of the administered 15N was recovered in the feces in the 3 days. Thus most of the administered 15N was absorbed by the body. The average 15N retention in the whole body on day 3 was 35.4%. Thus Papua New Guinea highlanders retained about one-third of the isotope administered as urea.

Figure 1 shows the changes of 15N atom% excess in urinary total nitrogen, urea and ammonia over 3 days. The values in the three fractions decreased exponentially.
Table 4. $^{15}$N atom% excess in non-protein N (NPN) and protein fractions in serum 3, 5 and 7 days after the administration of $[^{15}$N]urea to Papua New Guinea highlanders. (atom% excess)

| Subject | NPN$^a$ | Protein$^b$ |
|---------|---------|-------------|
|         | Day 3  | Day 3       | Day 5  | Day 7  |
|         | (10)$^c$ | (10)       | (3)    | (3)    |
| 1       | 0.12   | 0.008       | 0.012  |        |
| 2       | 0.19   | 0.012       |        |        |
| 3       | 0.03   | 0.002       | 0.006  | 0.006  |
| 4       | 0.17   | 0.006       |        |        |
| 5       | 0.06   | 0.011       | 0.006  | 0.005  |
| 6       | 0.14   | 0.005       |        |        |
| 7       | 0.05   | 0.018       |        |        |
| 8       | 0.01   | 0.004       |        |        |
| 9       | 0.24   | 0.004       |        |        |
| 10      | 0.08   | 0.012       | 0.003  |        |
| Mean    | 0.11   | 0.008       | 0.008  | 0.005  |
| ± SD    | 0.08   | 0.005       | 0.003  | 0.002  |

$^a$Measured by optical spectrographic analysis. $^b$Measured by mass spectrometric analysis. $^c$Number of samples.

with time. The value for urea was higher than that for ammonia and changed in parallel with that of urinary total nitrogen, indicating that most of the $^{15}$N in the urine was in urea. The isotope concentration in urinary ammonia was low, but the decay rate was less than that in urea.

Results on the $^{15}$N concentrations in serum NPN and protein are shown in Table 4. The value in serum NPN on day 3, with a large standard deviation, was 0.11 ± 0.08 atom% excess. The isotope concentration in serum protein, which was much lower than that in serum NPN, was 0.008 ± 0.005 on day 3, 0.008 ± 0.003 on day 5 and 0.005 ± 0.002 atom% excess on day 7. Since only three samples were obtained on days 5 and 7, it was impossible to evaluate the change in isotope concentration statistically. But the incorporation of the isotope into serum protein shows that Papua New Guinea highlanders utilize urea for protein synthesis in the body.

The correlations of $^{15}$N retention in the whole body and $^{15}$N incorporation into serum protein with certain variables are shown in Table 5. The correlation between $^{15}$N retention in the whole body and $^{15}$N incorporation into serum protein was significant ($r=0.54, p<0.05$). Both $^{15}$N retention in the whole body and $^{15}$N atom% excess in serum protein were correlated negatively with the daily average excretion of urinary nitrogen for the 3 days ($r=-0.69, p<0.005$ and $r=-0.54, p<0.05$), particularly with the urinary excretion during the early part of this period, but not...
Table 5. Correlations of $^{15}$N retention in the whole body and $^{15}$N atom% excess in serum protein in 3 days after administration of $[^{15}$N]urea with certain variables in Papua New Guinea highlanders.

$^{(n=10)^a}$

|                     | $^{15}$N retention in whole body | $^{15}$N atom% excess in serum protein |
|---------------------|---------------------------------|---------------------------------------|
|                     | $r$    | $p$          | $r$    | $p$          |
| $^{15}$N retention  |        |              |        |              |
| in whole body       |        |              | 0.54   | <0.05        |
| $^{15}$N atom% excess in serum NPN | -0.11 | NS$^c$       | -0.12  | NS           |
| Protein intake$^d$  |        |              |        |              |
| (g/kg body weight)  |        |              | -0.12  | NS           |
| Urinary N excretion |        |              | -0.24  | NS           |
| Day 1               |        |              | -0.52  | <0.05        |
| Day 2               |        |              | -0.55  | <0.05        |
| Day 3               |        |              | -0.30  | NS           |
| Mean$^e$            |        |              | -0.69  | <0.005       |
| Serum protein level |        |              | -0.48  | NS           |
| (g/100 ml)          |        |              | -0.63  | <0.01        |

$^a$ Number of subjects. $^b$ Values 3 days after administration of $[^{15}$N]urea. $^c$ Not significant. $^d$ Average in 3-day test period. $^e$ Average urinary nitrogen excreted on days 1, 2 and 3.

with the protein intake. Furthermore, the protein concentration in the serum was significantly negatively correlated with $^{15}$N incorporation into serum protein ($r = -0.63, p<0.01$), but not with $^{15}$N retention in the whole body. These results indicate that the subjects who excreted a smaller amount of urinary nitrogen tended to retain more $^{15}$N in the body and incorporate more $^{15}$N into serum protein, and that the subjects with lower level of serum protein tended to use more $^{15}$N derived from urea for the synthesis of serum protein.

DISCUSSION

There are two pathways of utilization of urea-N for protein synthesis: 1) One is release of urea into the gastrointestinal tract, its hydrolysis to ammonia by bacterial urease, use of the ammonia for bacterial protein synthesis and utilization of this protein as a dietary protein. 2) The other is conversion of urea to ammonia, absorption of this ammonia by the body, and then its conversion to various amino acids. It has been reported that dietary conditions influence the degree of ureolysis and urease activity in the intestinal tract ($^{17,18}$), but reported results are inconsistent. One report exists to the effect that the pattern of intestinal flora is influenced by the level of protein intake ($^{19}$). Benno $et$ $al.$ ($^{20}$) found, from examination of
stools, that Papua New Guinea highlanders consuming a low-protein diet, had a different intestinal flora with more ammonia-utilizing bacteria from that of Japanese men. This finding supports the possibility that Papua New Guinea highlanders have high ability to utilize urea-N.

The cumulative urinary excretion of $^{15}$N during 3 days as a percentage of the dose tended to be higher in the New Guinea highlanders studied than in the Japanese subjects on a low-protein diet (0.5 g/kg, for 2 weeks) reported by Tanaka et al. (13). This suggests that the New Guinea highlanders retained more $^{15}$N and thus utilized more urea-N in the body than Japanese subjects on a low-protein diet, though exact comparison of data on the two groups is difficult because of the difference in experimental backgrounds.

Although it is known that protein deficiency leads to high utilization of ingested urea-N, no significant correlation between the protein intake and the retention of $^{15}$N was observed in this study. However, urinary nitrogen excretion was found to be negatively correlated with $^{15}$N retention. The daily protein intake in individuals, which was not constant during the test period, may not have reflected their usual protein intake. The amount of urinary nitrogen excretion was affected by the level of protein intake before the test period as well as that during the test and thus was related to the status of protein metabolism in the whole body due to nutritional conditions before and during the test. This may be why $^{15}$N retention was correlated with urinary excretion, but not protein intake.

Previous studies showed that when $^{15}$N was administered as labeled urea or ammonium salt, its incorporation into body protein increased with the increase in its retention in the whole body (2). Similarly, in the present study, a significant positive correlation was observed between $^{15}$N retention and the $^{15}$N concentration in serum protein. It was also found that urinary nitrogen excretion was negatively correlated with $^{15}$N retention and the $^{15}$N concentration in serum protein. These results show that the subjects with a poor protein nutrition status, who showed low excretion of urinary nitrogen, utilized more urea-N for protein synthesis. This conclusion is supported by the observation that there was a negative correlation between the $^{15}$N concentration in serum protein and the serum protein level. Although the protein level of the serum in the highlanders was within the normal range for Japanese, the variation in this level appeared to be a true reflection of the status of protein nutrition.

There is some controversy about whether endogenous urea-N can really be utilized (27). However, Murdach (5) reported that during a period of protein restriction, the plasma protein of men and dogs became labeled with $^{15}$N when $[^{15}$N]urea was given parenterally. Furthermore, part of the urea produced in the body has been found to be released into the gastrointestinal tract to be hydrolyzed to ammonia, and $^{15}$N of the labeled urea and ammonia taken orally have been reported to be utilized for protein synthesis in the body (2). These findings seem sufficient evidence that endogenous urea-N is utilized. In addition, it is considered that even a trace level of $[^{15}$N]urea administered orally is metabolized in the same
way as endogenous urea released into the gastrointestinal tract. From these considerations and the results of utilization of $^{15}$N-urea in this study, we suggest that Papua New Guinea highlanders utilize endogenous urea-N and that its utilization is enhanced by the poor status of protein nutrition. This utilization is considered to be the result of a traditionally low protein intake. Moreover, in order to clarify the significant effect on Papua New Guinea highlanders of the utilization of urea-N for the adaptation to a low protein intake, it may be necessary to determine the absolute amount of urea-N utilized for protein synthesis.

The authors thank Dr H. Yoshimura, Kobe Women's College, Dr K. Sugimoto, Osaka City University, and Dr S. Hori and Mr J. Tsujita, Hyogo College of Medicine, for help in this study. They also thank Dr T. Mitsuoka, The Institute of Physical and Chemical Research, for analysis of $^{15}$N.

This study was supported by the Grants-in-Aid (304138 (1978) and 404340 (1979)) for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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