Effect of Dietary Protein Levels on Urea Utilization in Papua New Guinea Highlanders

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Abstract Ability to utilize urea nitrogen for body protein synthesis was examined with Papua New Guinea (PNG) highlanders and Japanese (JPN). Eight male PNG highlanders and 8 male JPN were fed on a low protein diet containing 0.55 g protein/kg or an adequate protein diet containing 1.34 g protein/kg for 1 or 2 weeks. The fate of 15N was measured after oral administration of 15N-labelled urea. There was no difference in 15N incorporation into serum protein between PNG highlanders and JPN receiving low protein diets. On the other hand, on the adequate protein diet, 15N incorporation in PNG highlanders was similar to that on the low protein diet, in contrast to that in JPN which was hardly detected in the adequate protein diet. When PNG highlanders take more protein than protein in their usual diet, they effectively incorporate ingested protein into their body protein and urea nitrogen is utilized for synthesis of body protein.

Key words: Papua New Guinea highlanders, 15N-urea metabolism, urea utilization, protein metabolism, potato eaters.

Many nutritionists in the world have recently become interested in nutritional problems in Papua New Guinea (PNG) (HIPSLEY and CLEMENTS, 1950; OOMEN, 1961, 1970; NORGAN et al., 1974; HIPSLEY, 1975). This is because PNG highlanders,
who live mainly on sweet potatoes, have a small physique but rarely show signs of malnutrition in spite of their very low protein intake. It is of particular interest to determine how the metabolism of these people changes nutritionally when they have a low protein diet.

Many investigations on PNG highlanders have already been carried out and reported. Hipsley (1975) proposed that they have adapted to long-term protein deficiency by maintaining a small physique and increasing the reutilization of urea. Oomen (1970) suggested that they have micro-organisms in the intestines which fix gaseous nitrogen.

In order to clarify the above problem, we have carried out a dietary survey, biochemical tests on the blood, examination of physical characteristics (Okuda et al., 1981) and a study of protein metabolism in the male highlanders of PNG (Fujita et al., 1986). On the dietary survey carried out in 1978, the highlanders’ daily protein intake was 0.6 g/kg body weight, which was about half of the Japanese recommended allowance (1980) (Okuda et al., 1981). Sweet potatoes contributed 47.6% of the total protein consumed.

Many studies have demonstrated that urea nitrogen is utilized in the synthesis of body protein in human subjects and animals on low protein intake (Walser and Bodenlos, 1959; Snyderman et al., 1962; Giordano, 1963; Richards et al., 1967; Giordano et al., 1968; Picou and Phillips, 1972; Varcoe et al., 1975; Long et al., 1978). In order to determine the subjects’ utilization of urea in reference to their food habits, we estimated the 15N nitrogen in serum protein after administering 15N-labelled urea to groups living among the PNG Eastern Highlands on habitual diets (Tanaka et al., 1980; Rikimar et al., 1985). 15N had been incorporated into serum protein on habitual diet 3 days after the administration of the 15N urea. But these were not investigated hourly for the first 3 days. We carried out experiments on the dietary nitrogen metabolism of PNG highlanders and of Japanese. We examined the difference in their ability to utilize urea by racial background and the level of protein intake, especially for the first 3 days.

MATERIALS AND METHODS

subjects. Studies were carried out in 1980 at the village of Beha near Lufa in the Eastern Highlands Province of PNG and on Osaka in Japan. Details of the PNG highlanders’ village and their way of living were reported elsewhere (Koishi et al., 1979; Okuda et al., 1981).

The subjects were 8 healthy male PNG highlanders and 8 healthy male Japanese 18 to 30 years of age, who were divided into 4 groups. The experiments (exps.) 1, 2, were made on PNG highlanders and exps. 3, 4, on Japanese subjects. The experimental protocol was approved by the Committee of Osaka City Univ. on the Use of Humans as Experimental Subjects. The full nature and purpose of the experiment were explained to these subjects and their consent was obtained before starting the study. The subjects gave normal values in urinary tests for sugar,
protein, acetone bodies and urobilinogen. The average height, body weight and ages of the subjects in each group are shown in Table 1. Experimental design is shown in Table 2. The subjects from the PNG highlanders lived in a metabolic ward which was built near our laboratory at the village and spent every day undertaking routine activities during the experimental period.

Diet. In PNG highlanders, a low protein diet (LPD) was given for 13 days and an adequate protein diet (APD) was given continuously for 18 days. In Japanese, LPD was given for 15 days and APD was given for 8 days respectively (Table 2).

LPD (exps. 1, 3) was provided by Japanese habitual food items. It was composed mainly of rice with mackerel, corned beef, eggs, and vegetables. Corn starch, sugar, and corn oil were supplied for energy compensation. LPD provided 0.5 to 0.6 g protein (22 to 30% of animal protein ratio) and 42 to 44 kcal of energy/
We adopted the level of 0.5 to 0.6 g/(kg·day) protein intake for these experiments. It may be considered low, but it is not necessarily deficient. This level was equivalent to PNG highlanders' habitual diet as described previously (Okuda et al., 1981).

APD (exps. 2, 4) was similar to the usual Japanese diet with respect to protein content. It provided 1.34 g protein (53% of animal protein ratio) and 42 to 44 kcal of energy/(kg of body weight)-day). This protein level was about twice that of the PNG highlanders' habitual diet or LPD. All diets were supplemented with an adequate daily allowance of vitamins and mineral mixtures.

For 4 days before the end of the experimental period, the subjects were given 20 mg/kg of urea labelled with 15N of 97.2 atom% excess orally at 6:00 a.m. before breakfast. Blood was withdrawn from the antecubital vein just before and 1, 6, 12, 24, and 72 h after administration of the 15N labelled urea. Subjects began to eat their diets 1 h after the administration. The serum was separated by centrifugation.

Urine was collected for 24 h periods for 4 days after 15N administration and volumes of urine were measured. Aliquot of serum and urine sample were frozen for transfer to Japan. Feces were marked by giving 3 g of charcoal powder to each subject. Feces were collected before 3 days and after 4 days of 15N administration. Feces were mixed with a little acidified water, dried at 95 to 100°C, weighed, and then ground to powder for transfer to Japan for analysis.

Blood: All measurements were checked by control serum (Ortho Diagnostics Inc., Raritan, New Jersey 08869).

Blood collection was carried out at the beginning of the experimental period. Serum total protein was determined by the biuret reaction method (Gornall et al., 1949), serum albumin by the bromcresol green method (Doumas et al., 1971) and serum urea by the indophenol method (Kaplan, 1970).

Urine and feces: The nitrogen contents of the urine and feces were determined by the semi-micro Kjeldahl method, and creatinine in the urine was measured by Folin's method as modified by Koishi (1962). From the results of the creatinine excretion in the urine of each subject, it seemed that the 24 h collections of urine were fairly precise.

Serum: The serum protein was treated with 20% trichloroacetic acid (TCA) and centrifuged (1,000 g × 15 min) to precipitate protein. The precipitate was washed three times with 5% TCA. This protein was digested and distilled by the semi-micro Kjeldahl method. At that stage, ammonium sulfate was submitted to 15N analysis.

The concentration of 15N in serum protein was analyzed by a mass spectrometer (Hitachi RMI-2).

Urine and feces: The urine and feces, in regard to ammonium sulfate were treated in the same way as the serum. This ammonium sulfate was diffused and
absorbed in 1 N-HCl and changed to NH₄Cl. The concentration of ¹⁵N in urine and feces was analyzed (using NH₄Cl) by emission spectrometry according to the method of Yamamura (1981).

The concentration of ¹⁵N was measured by the emission spectrometer (JACOS NIA-1).

RESULTS

¹⁵N excretion in urine and in feces

The percentage of ¹⁵N excretion in urine, feces and the total percentage of excretion and retention in the body are given in Table 3. The percentage of ¹⁵N excretion in daily urine was measured for 4 successive days after ¹⁵N administration. The cumulative curve of ¹⁵N in urine attained a maximum value in about 3 or 4 days and then reached a plateau. The ¹⁵N retention was calculated by subtracting the value of the ¹⁵N excretion in urine and feces for 4 days from the ¹⁵N dose. Fecal excretion of ¹⁵N was much less than ¹⁵N excretion in urine. Nearly all urea was excreted in urine.

The 83.5% of ¹⁵N urinary excretion in exp. 2 containing 1.34 g/kg protein in PNG highlanders was smaller than that in Japanese (exp. 4). Then ¹⁵N total excretion in exp. 2 (83.7%) was smaller than that in exp. 4 (95.7%). That in exp. 4 was significantly different from that in exp. 1 (70.3%) and exp. 3 (82.2%). In exp. 2, ¹⁵N retention was 16.4% of the ¹⁵N dose and it was higher than 4.2% of that in the same diet in Japanese (exp. 4), although it was not significant. In each diet, ¹⁵N retention in PNG highlanders was about 10% higher than that in Japanese.

¹⁵N in serum protein

Figure 1 shows ¹⁵N incorporation into serum protein after ¹⁵N administration. These values were the assayed values less background values which were measured

Table 3. ¹⁵N excretion in urine and feces after ¹⁵N oral administration.

<table>
<thead>
<tr>
<th>Exp. No. subject</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td></td>
<td>PNGH</td>
<td>PNGH</td>
<td>JPN</td>
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<tr>
<td></td>
<td>LPD</td>
<td>APD</td>
<td>LPD</td>
<td>APD</td>
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<tr>
<td>¹⁵N dose (mGN)</td>
<td>610 ± 24†</td>
<td>541 ± 55</td>
<td>577 ± 15</td>
<td>566 ± 50</td>
</tr>
<tr>
<td>¹⁵N fecal excretion (%) ††</td>
<td>0.9 ± 0.3a</td>
<td>0.2 ± 0.2b</td>
<td>0.6 ± 0.0a</td>
<td>0.4 ± 0.2a,b</td>
</tr>
<tr>
<td>¹⁵N urinary excretion (%)</td>
<td>69.4 ± 14.5a</td>
<td>83.5 ± 10.3a</td>
<td>81.7 ± 3.6a</td>
<td>95.3 ± 3.5b</td>
</tr>
<tr>
<td>¹⁵N total excretion (%)</td>
<td>70.3 ± 14.3a</td>
<td>83.7 ± 10.2a,b</td>
<td>82.2 ± 3.6a</td>
<td>95.7 ± 3.3b</td>
</tr>
<tr>
<td>¹⁵N retention (%)</td>
<td>29.8 ± 14.3a</td>
<td>16.4 ± 10.2a,b</td>
<td>17.8 ± 3.6a</td>
<td>4.2 ± 3.3b</td>
</tr>
</tbody>
</table>

PNGH, Papua New Guinea highlanders; JPN, Japanese; LPD, low protein diet; APD, adequate protein diet. † mean ± S.D. a,b Means in same row sharing a common superscript letter are not significantly different (p<0.05). †† Percentage of administrated ¹⁵N excreted over 4 days.

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Fig. 1. $^{15}$N incorporation into serum protein. Each value shows the concentration of $^{15}$N in serum protein less the background level measured just before $^{15}$N urea administration. PNGH, Papua New Guinea highlanders; JPN, Japanese; LPD, low protein diet; APD, adequate protein diet. *Significantly different from APD in Japanese (p<0.05). Bars indicate the values of S.D.
just before (0 h). \(^{15}\text{N}\) concentration in serum protein at 1 h was 16.0±5.8 atom\(\%\) on LPD, 12.4±3.2 atom\(\%\) on APD in PNG highlanders, and 13.3±5.2 atom\(\%\) on LPD, 2.3±2.4 atom\(\%\) on APD in Japanese. That of APD in Japanese was significantly lower than those of others. There was already the maximum \(^{15}\text{N}\) incorporation which appeared in protein 1 h after \(^{15}\text{N}\) administration, and then the enrichments remained nearly constant for the following 72 h. This \(^{15}\text{N}\) which was administrated as urea \(^{15}\text{N}\) was shifted to some other amino acids and then was incorporated into protein. On LPD, the \(^{15}\text{N}\) concentration in serum protein in PNG highlanders and Japanese was on the same level. But on APD, that in PNG highlanders kept a higher level than that in Japanese throughout the experimental period, and in fact it was similar to that on LPD in PNG highlanders. In comparison with PNG highlanders the \(^{15}\text{N}\) on APD in Japanese was scarcely incorporated into serum protein.

**DISCUSSION**

A number of suggestions as to how PNG highlanders adapt to a low intake of nutrients have been made. OOMEN (1970) examined 23 highlanders and reported that they were in severe negative N-balance of about 2 g/day, and that fecal nitrogen excretion was very large. He therefore suggested that some micro-organisms might exist in their intestines which fixed gaseous nitrogen and thus compensated for their low protein intake. HIPSLEY (1975) suggested the possibility of reutilization of urea nitrogen, being incorporated into body protein. In the past, many nutritional surveys have been carried out (HIPSLEY and Clements, 1950; OOMEN, 1961; NORGAN et al., 1974). On the survey carried out as part of our current study, we did not find any very low protein intake or the severe negative N-balance that had been reported previously (KAIWARA et al., 1984; OKUDA et al., 1985; FUJITA et al., 1986). They kept zero balance but OOMEN (1970) indicated −2 gN/day balance. However, it was clear that PNG highlanders' protein intake was about half of the usual average of Japanese intake. Thus, we paid attention to their protein metabolism, especially their ability to utilize urea.

In order to determine the degree of urea utilization and to make clear the role of urea in nitrogen metabolism, the fate of \(^{15}\text{N}\) labelled urea has been studied in plasma protein by many investigators. GIORDANO (1963) and GIORDANO et al. (1968) reported that in healthy subjects urea nitrogen was more easily utilized for synthesis of amino acids when protein intake was very low. They also pointed out that a larger amount of urea was used for synthesis of nonessential amino acids in patients with chronic renal failure when the protein intake was similarly small. This finding was also confirmed by RICHARDS et al. (1967) and VARCOE et al. (1975).

Recently Namioka and his coworkers examined urea utilization by using \(^{15}\text{N}\) urea in pigs, which are monogastric and which are omnivorous (DEGUCHI et al., 1978, 1980; NIYAMA et al., 1979). It was demonstrated that at first urea was broken down to ammonia by intestinal flora, and then amino acids were synthesized by
micro-organisms from this ammonia. These amino acids entered the portal vein and were utilized for body protein synthesis in the pig. Ammonia was also absorbed immediately from the intestines, incorporated into non-essential amino acids in the liver and utilized in the synthesis of body protein in the pig. Similar results were reported in other experimental animals. It was reported that germ-free animals could not break down urea to ammonia, therefore they could not utilize urea for body protein synthesis (KOBASHI, 1979).

We have demonstrated that urea is utilized for synthesis of body protein by finding incorporation of $^{15}$N from urea into serum protein. We compared the degree of urea utilization between healthy PNG highlanders and Japanese subjects in relation to level of protein intake. TANAKA et al. (1980) and RIKIMARU et al. (1985) tried to make clear the mechanism of the utilization of urea in PNG highlander’s diet which was composed mainly of sweet potatoes. The protein level on that diet was the same as with LPD in this experiment. And $^{15}$N incorporation into serum protein in their habitual diet was the same as with LPD. On low protein intake, whether sweet potato diet or rice diet, PNG highlanders similarly utilized urea nitrogen. There was no difference with foodstuff. TANAKA et al. (1980) estimated $^{15}$N incorporation into hydrolysate of serum protein until 10 days on their habitual diet. $^{15}$N was incorporated into non-essential amino acids and essential amino acids, even lysine. The PNG highlanders had the blood sample collected after the third day or daily of $^{15}$N labelled urea administration on their habitual diet mentioned above. The level of $^{15}$N in the serum protein was already high in all samples. The result obtained on their habitual diet suggested that the highest level of $^{15}$N incorporation was detected for the first 3 days. Then in the present experiment we collected the blood samples on 1, 6, 12, 24, and 72 h after $^{15}$N urea administration, and tried to make clear the detail of $^{15}$N incorporation into serum protein. The highest $^{15}$N incorporation into serum protein appeared at an earlier hour (1 or 6 h). It was suggested, as urea was easily utilized in the body, $^{15}$N in urea was incorporated into serum protein immediately. It is established that urea is utilized for body protein synthesis on low protein intake, but on adequate protein intake urea is hardly utilized (GIORDANO et al., 1968; VARCOE et al., 1975). In fact, it was so with Japanese (Fig. 1). But it was noticed that, when PNG highlanders ingested adequate protein intake, they utilized urea on the same level as with low protein intake. These results indicate that PNG highlanders utilize urea to the same extent when the level of protein intake was either high or low. With reference to $^{15}$N excretion in urine and feces, PNG highlanders on APD retained 16% of the $^{15}$N dose in the body, whereas Japanese subjects on APD excreted almost the whole dose.

Fujita et al. (1986) reported N-balance data in the same experiment in this paper. When PNG highlanders are their habitual diet or a low protein diet (LPD), they maintained about zero balance. In contrast, when PNG highlanders ate APD, the N-balance was +45.1 mgN/(kg-day) and remarkable nitrogen accumulation was observed. SMITH et al. (1974) found persistently positive N-balance after 80
days' refeeding time in malnourished patients. The refeeding diet contained 154.4 g protein/day. By that time, the patients had become normal in every other respect. When PNG highlanders take much more protein than that in their usual diet, they still showed utilization of urea, which is consistent with the finding of the high positive nitrogen balance on an adequate protein diet. And in these two kinds of diets, percentage of $^{15}$N retention was some 10% higher with PNG highlanders than with Japanese (Table 3). As urea retention in the body in PNG highlanders was higher, it was suggested that urea might be utilized more often.

Fujita et al. (1986) also reported that fecal nitrogen was 1.3-1.4 times higher in their habitual sweet potato diet than in a rice diet with both PNG highlanders and Japanese. These results might be related to the different amount of dietary fiber in these diets. Total amount of crude fiber on LPD and APD was one-third that of their habitual diet. Mizutani et al. (1983) reported that the amount of dietary fiber also affect the pattern of the intestinal flora. Then urease-producing bacteria may growth. This may affect urea metabolism. Benno et al. (1983) reported that intestinal flora in PNG highlanders was different from that in Japanese. Oomen (1970) suggested that intestinal flora might fix gaseous nitrogen and allow it to be incorporated into body protein, but Benno et al. (1983) could not find any nitrogen-fixing organism in the feces of PNG highlanders. This fact requires further investigation.

In conclusion, all groups except the Japanese on APD effectively utilized ingested urea nitrogen for serum protein synthesis. The Japanese were capable of utilizing urea nitrogen when they took a low protein diet. PNG highlanders utilized urea nitrogen in their usual diet. The $^{15}$N concentration in serum protein of PNG highlanders was equally high when they ingested an adequate protein diet. When PNG highlanders took more protein than that in their usual diet, they were able to utilize urea nitrogen too. They effectively incorporate ingested protein into their body protein (Fujita et al., 1986) and urea nitrogen is utilized for synthesis of body protein. It may be the reason why they have a small physique but rarely show signs of malnutrition in spite of low protein intake.

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