Blood Status and Serum Free Amino Acids in Papua New Guinea Highlanders

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Summary Hematological values and serum amino-acid concentrations were measured in 17 healthy male adult Papua New Guinea highlanders who live on a sweet-potato staple diet. Hematological values were within the normal range, except for a low serum urea concentration. The concentrations of serum threonine, valine, isoleucine, leucine and tyrosine were significantly lower, and those of arginine, glutamate, glycine and alanine were significantly higher, than in Japanese controls. These amino acid patterns in the serum of Papua New Guinea highlanders are an indication of low protein nutrition and adequate energy supply. Some essential amino acid and urea concentrations in the serum of nine Papua New Guinea subjects fed on an adequate protein diet (1.3 g/kg body weight, about twice their habitual diet) for 13 days were significantly increased but were still significantly lower than those of Japanese subjects. Serum alanine decreased on an adequate protein diet. These results show that amino acid uptake and utilization by peripheral tissues may be accelerated on an adequate protein diet. Blood status and serum amino acid

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concentrations did not show any change except for urea and some amino acids, when five Papua New Guinea highlanders were fed on a low protein diet (0.6g/kg) consecutively for 13 days.

**Key Words** serum amino acid, blood status, dietary protein, protein nutrition, Papua New Guinea highlanders

Dietary protein deficiency is a problem widespread in developing countries. In order to clarify the mechanism for adaptation to protein deficiency in Papua New Guinea (PNG) highlanders, we have been carrying out nutritional studies in Goroka and in the village of Beha in the Eastern Highlands Province of Papua New Guinea since 1977 (1–4). Low protein intake has been recognized as a common condition throughout the highlands of Papua New Guinea, where the staple diet is sweet potato (2, 5, 6).

We carried out a nutritional survey of 18 healthy adults in August, 1978 in the village of Beha (2). The mean daily energy intake was 2,390 ± 540 kcal, which was about as much as the daily energy expenditure. The daily protein intake was 35.2 ± 10.7 g (0.6 g/kg body weight) and sweet potatoes and other tubers accounted for 47.6% of the total protein consumed. These results are similar to those of the dietary surveys carried out in the Papua New Guinea highlands in the past by others (5, 6), except that highlanders now include some store-bought foods such as rice and canned fish in their diet. It is noteworthy that the growth rate of children and the adults’ physique were almost similar to those of Japanese, except that they were short in stature, despite the poor quantity and quality of protein in their food. Their nitrogen balance was slightly negative, but not as negative as that reported by Oomen (5). From our studies using $^{15}$N urea, it has been shown by the oral administration of $^{15}$N in the form of urea that urea-N can be utilized for plasma protein synthesis in PNG highlanders (1).

Although a number of studies have shown the influence of low-protein diets on serum amino acids, this has not been reported for PNG highlanders. This paper reports serum free amino acid patterns in PNG highlanders and also describes the effects on these patterns of dietary protein level and the intake of different kinds of non-habitual foodstuffs.

**METHODS**

**Description of the area.** We carried out nutritional experiments in Beha village in Eastern Highlands Province during October–December, 1980. The village of Beha is located on the north-west side of Mt. Michael and at an altitude from 1,500 m to 2,100 m. The staple food consists mainly of sweet potatoes, with taro and yam as important subsidiaries. These tubers have a low protein content varying from 0.6 to 1.7%. Green leaves, bananas and beans are also eaten, but they tend to be taken seasonally. Beha people eat pork only on special occasions, such as festivals.
celebrations of births, marriages, funerals, etc. Recently, Beha people have started to buy food, particularly rice and canned foods, using money earned from their coffee gardens. But the use of such foods is infrequent and the amount eaten is small, though it is increasing. None of the subjects of our dietary survey in 1978 and 1980 were found eating eggs or milk. Further descriptions of this area have been given elsewhere (2, 4).

Subjects. The Japanese subjects were 7 healthy male university students aged 20 to 23 years. The Papua New Guinea highlanders were 17 healthy males ranging from 18 to 40 years of age and they gave normal values on urinary tests for sugar, protein, acetone bodies and urobilinogen. The physical characteristics of the Japanese and PNG subjects are given in Table 1. Nine of the PNG subjects lived in a bush house near our laboratory and ate the experimental diet under our clinical supervision for the entire duration of the experiment. The purpose and methods of the proposed studies were fully explained to each subject. The procedures followed were in accordance with the Helsinki Declaration as updated in Tokyo, Japan 1975 and with the approval of the Faculty Council of Osaka City University.

Diets. In the first period of 13 days, nine PNG subjects were fed on an adequate protein diet (1.3 g/kg, 53% of animal protein ratio), which contained about twice as much protein as their habitual diet (7) and was similar to a Japanese daily intake. Five of them were then fed on a low-protein diet (0.6 g/kg, 22% of animal protein ratio) for the next 13 days. Experimental diets were composed of rice, potatoes, canned mackerel, corned beef, dried eggs, leaves, etc., and these differed from the habitual diet. Most foodstuffs were used with some variety every day during the experimental period. In the case of the low-protein diet, corn starch, sugar and corn oil were supplied for energy compensation. All food items were accurately weighed to 1 g on a balance prior to being served.

The mean daily intake of nutrients during the whole experimental period is

| Subject | Japanese | | PNG |
|---------|----------|----------|---------|
| Diet    | Habitual | Habitual | Adequate protein | Low protein |
| Duration (day) | — | — | 13 | 13 |
| Protein level (g/kg) | (1.3)$^1$ | (0.73)$^2$ | 1.35 | 0.61 |
| No. of subjects | 7 | 17 | 9 | 5 |
| Age (yr) | 21 ± 1$^3$ | 28 ± 5 | 26 ± 5 | 27 ± 5 |
| Height (cm) | 169.0 ± 6.4 | 161.2 ± 5.4 | 161.3 ± 6.3 | 162.3 ± 3.8 |
| Body weight, initial (kg) | 58.1 ± 5.1 | 60.2 ± 5.9 | 60.7 ± 5.1 | 64.4 ± 3.5 |
| Body weight, final (kg) | — | 61.2 ± 5.6 | 64.2 ± 3.6 |

$^1$ National Nutrition Survey by the Ministry of Health and Welfare of Japan (1981).
$^2$ Nutrition survey (1980) (7). $^3$ Mean ± SD.
given in Table 2. Energy intake during the experiment was regulated to maintain constant body weight. The diets were supplemented with three tablets of vitamins and 1 g of mineral mixtures. A vitamin tablet (courtesy of Takeda Chemical Industries, Ltd.) contained 1,000 IU retinol palmitate, 100 IU ergocalciferol, 5 mg fursultiamine, 2 mg riboflavin, 3 mg pyridoxine hydrochloride, 25 mg niacinamide, 5 μg cyanocobalamin, 75 mg ascorbic acid, 5 mg tocopherol acetate, 15 mg calcium pantothenate, 46.8 mg precipitated calcium carbonate and 34 mg dibasic calcium phosphate. One hundred grams of mineral mixture contained 39.25 g KHCO₃, 25.11 g CaCO₃, 21.74 g KH₂PO₄, 10.78 g MgSO₄·7H₂O, 2.91 g FeC₆H₅O₇·6H₂O, 0.1314 g CuSO₄·5H₂O, 0.0366 g MnSO₄·4H₂O, 0.0316 g ZnCl₂ and 0.0067 g KI. The experimental diets contained 4–5 times the crude fat and one-third the crude fiber of the habitual diets (Table 2). Subjects were fed at 7:00 h, 12:00 noon and 18:00 h.

Blood collection was carried out at the end of the experimental period. Blood samples were obtained from the antecubital vein in the postabsorptive state, after at least 12 h of fasting. The hematocrit was measured by high-speed centrifugation in a capillary tube (8). Hemoglobin was measured by the international standard method (9). The circulating plasma volume was determined by the dilution method with Evans blue (10) and the circulating blood volume was calculated using the hematocrit value. Serum specimens were then frozen and were transported in an ice box at −20°C by plane to Japan. Serum total protein was determined by the biuret reaction method (11) and serum albumin with the bromcresol green method (12). Globulin level was calculated by difference. Serum urea was determined by the indophenol method (13).

For the analysis of amino acids, 1.0 ml of serum was deproteinized with 5.0 ml of 1% picric acid and 4.2 ml of the protein-free supernatant fluid was applied to a Dowex 2 × 8 column (Cl⁻ form, 20–50 mesh, 0.7 cm × 7.0 cm), followed by 7.0 ml of 0.02 M hydrochloric acid. The effluent was lyophilized and the residue dissolved in redistilled water. Serum amino acids were determined by ion-exchange chromatography with two columns of an amino acid analyzer (14), Model LC-SS of Japan.

Table 2. Intake of nutrients for experimental diets.

| Diet | Habitual¹ | Adequate protein | Low protein |
|------|-----------|------------------|------------|
| No. of subject | 8 | 9 | 5 |
| Energy (kcal/kg) | 47.5 ± 7.8² | 43.6 ± 0.9 | 42.7 ± 1.0 |
| Protein (g/kg) | 0.73 ± 0.20 | 1.35 ± 0.02 | 0.61 ± 0.01 |
| Animal protein (%) | 17.1 ± 13.4 | 52.7 ± 0.7 | 21.8 ± 0.3 |
| Crude fat (g) | 16.5 ± 5.6 | 65.0 ± 4.2 | 84.9 ± 4.1 |
| Crude fiber (g) | 14.2 ± 4.7 | 5.2 ± 0.5 | 5.5 ± 0.3 |
| Carbohydrate (g) | 630 ± 114 | 420 ± 29 | 457 ± 15 |

¹ Nutrition survey (1980) (7). ² Mean ± SD.
Electron Optics Laboratory. Sodium citrate buffer was used for the determination of basic amino acids and lithium citrate buffer for acidic and neutral amino acids in a three-buffer system with temperature change.

Statistical methods. Values are expressed as mean ± SD. Student’s t test was used for comparing means.

RESULTS AND DISCUSSION

Blood status of PNG subjects on habitual diets

Table 3 shows the hematological data. Except for serum urea concentration, the results of blood and serum analysis in PNG subjects on a habitual diet were within the normal range. Hematocrit and hemoglobin values in PNG subjects in spite of their low protein intake were 47.1 ± 2.9% and 16.3 ± 1.1 g/100 ml, respectively, and were similar to those of Japanese subjects.

The concentration of total serum protein of PNG subjects was 8.13 ± 0.81 g/100 ml and was significantly higher than that of Japanese subjects. These results are similar to the data given in other reports on PNG highlanders (1, 3, 4, 6) and give no indication of protein-energy deficiency. The serum albumin level in PNG subjects was significantly lower than that of Japanese subjects; the globulin level was twice as high. Itoh et al. (3) reported that the concentration of gamma-globulin in the people of this area was significantly higher than that of Japanese, as determined by cellulose acetate membrane electrophoresis. Previous work carried out in Papua New Guinea also indicates that elevated globulin levels are invariably due to an increased gamma-globulin fraction (15). The reason for the change in serum protein concentration is not clear. The activities of GOT, GPT, LDH, cholinesterase, γ-GTP, alkaline phosphatase and leucine amino peptidase in serum were normal (unpublished data), and the subjects were

Table 3. Blood status of Japanese and PNG subjects.

| Subject          | Japanese | PNG          |             |             |
|------------------|----------|--------------|-------------|-------------|
|                  | Diet     | Habitual     | Habitual    | Adequate protein | Low protein |
| No. of subjects  | 7        | 17           | 9           | 5            |
| Hematocrit (%)   | 46.7 ± 2.5a | 47.1 ± 2.9a  | 46.3 ± 2.1a | 47.7 ± 3.0a  |
| Hemoglobin (g/100 ml) | 15.5 ± 1.1a | 16.3 ± 1.1ab | 16.5 ± 0.5b | 17.3 ± 1.3b |
| Serum protein (g/100 ml) | 7.25 ± 0.09a | 8.13 ± 0.81b | 8.01 ± 0.57b | 8.29 ± 0.60b |
| albumin (g/100 ml) | 4.93 ± 0.25a | 4.13 ± 0.33b | 4.29 ± 0.35bc | 4.48 ± 0.27c |
| globulin (g/100 ml) | 2.33 ± 0.19a | 4.00 ± 0.67b | 3.72 ± 0.55b | 3.82 ± 0.40b |
| urea (mg N/100 ml) | 16.1 ± 2.9a | 5.61 ± 1.61b | 11.3 ± 3.1c  | 4.80 ± 0.98b |

1 Mean ± SD; means within line not sharing a common superscript letter differ significantly (p < 0.05).

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physically healthy. The albumin concentration might be affected by the level of protein intake and the globulin concentration might be congenitally high.

PNG subjects who ate habitual diet had a serum urea nitrogen level of $5.61 \pm 1.61$ mg/100 ml, which was about one-third that for Japanese controls and at about the level found in Japanese fed on a low-protein diet containing 0.6 g/kg of protein (unpublished data). This low level of serum urea nitrogen may be due to their low protein intake. Eggum shows that there is a positive correlation between the protein content of the diet and blood urea concentration in animals (16).

Effect of dietary protein level on blood status
The serum urea concentration of PNG subjects fed on the adequate protein diet was doubled but was still significantly lower than that of the Japanese subjects (Table 3). PNG subjects fed on the adequate protein diet showed high positive nitrogen balance. The latter might depend on low levels of serum urea. The protein level in the diet had no effect on hematocrit value, concentration of hemoglobin, serum protein or albumin, although this low-protein diet differed very much in nutritional composition from the habitual diet of the PNG subjects, especially as regards crude fat, crude fiber and the animal protein ratio. Also, no definite effect on blood status was observed when PNG subjects were fed on the low-protein diet. A period of 13 days is probably too short a time period to see changes in the hematological parameters measured.

Circulating blood volume
The mean values for the circulating plasma volume and blood volume in the postabsorptive state of PNG subjects were $3,428 \pm 428$ ml ($56.3 \pm 5.1$ ml/kg) and $5,948 \pm 775$ ml ($97.5 \pm 8.2$ ml/kg) (Table 4). These values are comparable to those observed by Fujita in young Japanese men fed on an adequate protein diet (17). This result indicates that it was not diminution of their blood volume that determined the normal hematocrit and hemoglobin concentration of PNG subjects and that the serum protein level was genuinely high in spite of the low protein intake.

Serum free amino acids in PNG subjects
On the habitual diet. The aminogram obtained in Japanese controls is comparable with those reported by others (17, 18) (Table 5). Five of the essential

Table 4. Circulating blood volume of PNG subjects.

|                      |                   | 5                  |
|----------------------|-------------------|--------------------|
| No. of subject       |                   | 5                  |
| Body weight (kg)     | 61.0 \(\pm\) 5.5^1|                   |
| Plasma volume (ml)   | 3,428 \(\pm\) 428  |                   |
| (ml/kg)              | 56.3 \(\pm\) 5.1   |                   |
| Blood volume (ml)    | 5,948 \(\pm\) 775  |                   |
| (ml/kg)              | 97.5 \(\pm\) 8.2   |                   |

^1 Mean \(\pm\) SD.
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Table 5. Amino acid concentrations in serum (μM).

| Subject    | Japanese     | PNG          |
|------------|--------------|--------------|
| Diet       | Habitual     | Adequate protein | Low protein |
| No. of subjects | 7            | 17           | 9           | 5           |
| Lysine     | $204 \pm 33^{\text{a}}$ | $223 \pm 29^{\text{ab}}$ | $237 \pm 25^{\text{b}}$ | $228 \pm 20^{\text{ab}}$ |
| Threonine  | $132 \pm 18^{\text{a}}$ | $104 \pm 15^{\text{b}} (8)^2$ | $123 \pm 14^{\text{a}}$ | $93 \pm 10^{\text{b}}$ |
| Valine     | $251 \pm 36^{\text{a}}$ | $174 \pm 28^{\text{b}}$ | $217 \pm 21^{\text{c}}$ | $203 \pm 21^{\text{c}}$ |
| Methionine | $20 \pm 4^{\text{a}}$ | $21 \pm 7^{\text{a}}$ | $12 \pm 6^{\text{b}}$ | $6 \pm 3^{\text{b}}$ |
| Isoleucine | $84 \pm 15^{\text{a}}$ | $61 \pm 9^{\text{b}}$ | $74 \pm 8^{\text{a}}$ | $70 \pm 6^{\text{b}}$ |
| Leucine    | $153 \pm 26^{\text{a}}$ | $108 \pm 16^{\text{b}}$ | $126 \pm 14^{\text{c}}$ | $127 \pm 10^{\text{ac}}$ |
| Tyrosine   | $65 \pm 9^{\text{a}}$ | $52 \pm 10^{\text{b}}$ | $50 \pm 6^{\text{b}} (8)$ | $52 \pm 4^{\text{b}}$ |
| Phenylalanine | $65 \pm 10^{\text{a}}$ | $57 \pm 12^{\text{a}}$ | $59 \pm 11^{\text{a}} (8)$ | $63 \pm 5^{\text{a}}$ |
| Histidine  | $98 \pm 10^{\text{b}}$ | $99 \pm 21^{\text{ab}}$ | $88 \pm 15^{\text{a}}$ | $107 \pm 11^{\text{b}}$ |
| Arginine   | $75 \pm 12^{\text{a}}$ | $132 \pm 19^{\text{b}}$ | $105 \pm 8^{\text{a}}$ | $111 \pm 12^{\text{ed}}$ |
| Serine     | $106 \pm 18^{\text{a}}$ | $92 \pm 25^{\text{a}} (7)$ | $115 \pm 7^{\text{a}}$ | $115 \pm 7^{\text{a}}$ |
| Glutamate  | $60 \pm 13^{\text{a}}$ | $174 \pm 64^{\text{b}} (7)$ | $299 \pm 96^{\text{c}}$ | $288 \pm 86^{\text{c}}$ |
| Proline    | $175 \pm 32^{\text{a}}$ | $202 \pm 45^{\text{a}}$ | $176 \pm 28^{\text{a}} (8)$ | $190 \pm 35^{\text{a}}$ |
| Glycine    | $244 \pm 31^{\text{a}}$ | $348 \pm 68^{\text{b}}$ | $384 \pm 68^{\text{be}} (8)$ | $437 \pm 38^{\text{c}}$ |
| Alanine    | $351 \pm 66^{\text{a}}$ | $505 \pm 106^{\text{b}}$ | $379 \pm 72^{\text{a}}$ | $603 \pm 72^{\text{a}}$ |

1 Mean ± SD; means within line not sharing a common superscript letter differ significantly ($p<0.05$). 2 Figures in parentheses refer to number of subjects.

amino acids, threonine, valine, isoleucine, leucine and tyrosine, were significantly lower in the serum of PNG subjects than in Japanese subjects. In particular, the concentrations of valine and leucine in the serum were low (about 70% of Japanese control values). Swendseid et al. reported that of all the essential amino acids in plasma, valine appeared to be reduced to the greatest extent during trials with a low-protein diet (19).

Of the nonessential amino acids, arginine, glutamic acid, glycine and alanine were significantly higher in PNG subjects than in Japanese subjects. Although the serum level of alanine might increase in normal adults on isoenergetic, low-protein diets (17, 19), if severe energy, as well as protein, deficiency is present such an increase is less likely (20) because the hepatic capacity for conversion of alanine to glucose exceeds that of all other amino acids (21). The high level of alanine in PNG subjects indicates that they took in adequate energy from their habitual diet. The serum level of glutamic acid was about three times the level found in Japanese subjects. There was no difference in serum concentrations of histidine, serine and proline between the two groups.

The total essential amino acid level in the serum of PNG subjects was lower and the total nonessential amino acid level much higher than those of Japanese
Table 6. Total and ratio of serum amino acid levels.

| Subject                        | Japanese          | PNG               |
|--------------------------------|-------------------|-------------------|
|                                | Habitual          | Habitual          | Adequate protein | Low protein |
| No. of subjects                | 7                 | 7                 | 8                | 5           |
| EAA total (mm)                 | 0.98 ± 0.12¹a     | 0.82 ± 0.08bc     | 0.90 ± 0.06bc    | 0.84 ± 0.04c |
| NEAA total (mm)                | 1.11 ± 0.12a      | 1.57 ± 0.18b      | 1.55 ± 0.17b     | 1.85 ± 0.07c |
| Grand total (mm)               | 2.09 ± 0.23a      | 2.40 ± 0.19b      | 2.45 ± 0.17b     | 2.69 ± 0.04c |
| E/N ratio                      | 0.88 ± 0.06a      | 0.53 ± 0.08bc     | 0.58 ± 0.08b     | 0.46 ± 0.04c |
| Valine/glycine                 | 1.04 ± 0.14a      | 0.52 ± 0.11b      | 0.58 ± 0.10b     | 0.47 ± 0.08b |
| Leucine/alanine                | 0.44 ± 0.08a      | 0.23 ± 0.07b      | 0.34 ± 0.07c     | 0.21 ± 0.03b |
| Branched-chain amino acids/alanine | 1.42 ± 0.23a     | 0.71 ± 0.18b     | 1.12 ± 0.20c     | 0.67 ± 0.11b |

¹ Mean ± SD; means within line not sharing a common superscript letter differ significantly (*p* < 0.05).

subjects (Table 6). Whitehead proposed that the serum amino acid pattern was influenced by diet and the ratio of the essential to the nonessential amino acids (E/N ratio) in serum might be of value as an indicator of protein nutrition (22). The E/N ratio was 0.53 ± 0.08 for PNG subjects and 0.88 ± 0.06 for Japanese subjects. Arroyave reported that the estimation of the ratio between selected amino acids was more promising than the E/N ratio as an index of inadequate protein intake (23). Valine to glycine, leucine to alanine and branched-chain amino acids to alanine were calculated. All these indices of PNG subjects were significantly lower than those of Japanese. However, both essential and nonessential amino acid concentrations in the serum of PNG subjects were strikingly high as compared with values obtained in adults with protein-energy deficiency (indicated by severely diminished muscle mass, edema and hypoalbuminemia) (24). Thus, these amino acid patterns in the serum of PNG subjects indicate low protein nutrition and adequate energy supply. This view is supported by the dietary survey (2).

On the adequate protein diet. The concentrations of threonine, valine, isoleucine, leucine and glutamate in the serum of PNG subjects fed on the adequate protein diet were significantly greater than those of PNG subjects fed on their habitual diet (Table 5). However, the levels of increase in essential amino acids were low (120–130%). The concentrations of methionine, arginine and alanine decreased in subjects on the adequate protein diet. Anderson showed that by changing the protein intake of rats from low to high, the concentration of total amino acids rose markedly over the first few days and he suggested that this was probably due to the low activities of the enzymes of amino acid catabolism (25). Thereafter, following an increase in the activity of these enzymes, plasma amino acid concentrations decreased and urea synthesis and excretion increased (25). On the other hand, the J. Nutr. Sci. Vitaminol.
branched-chain amino acids in the plasma of rats fed on high-protein diets continued to increase with time while all other amino acids decreased (25). In this study, if blood collection could have been carried out on the first few days after feeding on the adequate protein diet, the amino acid concentration in serum may have been seen to have greatly increased. Unfortunately, blood was drawn only 13 days after the subjects had started on the adequate protein diet. The concentrations of urea and the branched-chain amino acids in serum and the excretion of urinary nitrogen (unpublished data) increased 13 days after the start of the adequate protein diet. These results may show a trend towards adaptation to high protein intake from the habitual low-protein diet. Since the level of increase of essential amino acids in serum was not high and alanine and arginine levels in serum decreased, it seems that these results were caused by the increased catabolism of amino acids. However, on the adequate protein diet, all subjects exhibited a greatly positive nitrogen balance, $+45.1 \pm 19.3 \text{mg N/kg}$, for the last 4 days and the utilization for serum protein of urea nitrogen in spite of an adequate protein intake (unpublished data). Serum alanine decreased on the adequate protein diet. Alanine is quantitatively the primary amino acid released by muscle, small intestine and other tissues in the postabsorptive state (21). In addition, valine, methionine, leucine, tyrosine and urea levels of PNG subjects fed on the adequate protein diet were significantly lower than those of Japanese, although the amount of protein intake was almost the same in the two groups. These results therefore show that amino acid uptake and utilization for protein synthesis by peripheral tissues may be accelerated in PNG subjects fed on an adequate protein diet which contains much more protein than the habitual diet. The E/N ratio in serum did not differ in those subjects on an adequate protein diet from those on a habitual diet, but the branched-chain amino acids/alanine ratio was significantly different and thus protein metabolism in the body might be different in the two groups.

Nitrogen balance and amino acid balance across forearm tissues were studied by Smith et al. in adult patients with severe protein-energy deficiency (24). Nitrogen balance was initially $-2.4 \text{g/day}$ on a $2 \text{g/day}$ nitrogen intake and became $+6.6 \text{g/day}$ on a $24.7 \text{g/day}$ intake after re-feeding for a period of 2 or 3 months. Both during malnutrition and after the re-feeding period, postabsorptive plasma arterio-venous differences in several amino acids were small compared with values in normal Americans (24). The authors suggested that persistently subnormal amino acid release from peripheral tissues probably restricted the supply of amino acids to the liver for gluconeogenesis and that this played a role in overall nitrogen conservation (24).

On the low-protein diet. The level of threonine in serum decreased and the levels of histidine and alanine were increased in five PNG subjects fed on the low-protein diet for 13 days after the adequate protein diet (Table 5). Other amino acid concentrations in PNG subjects fed on the low-protein diet did not change compared to the levels while on the adequate protein diet. Some of them did not return to the level of the habitual diet, although the amount of protein intake was
almost the same for the low-protein and the habitual diet. This difference may be due to differences in the amount of intake in dietary fiber and fat or in dietary history just before the experimental period of the low-protein diet. The low-protein diet contained only one-third of the level of crude fiber in the habitual diet. The digestibility of protein in the low-protein diet estimated from fecal nitrogen analysis in the same subjects was much higher than in the habitual diet (unpublished data). High digestibility may contribute to the high level of valine and leucine found in serum. Swendseid et al. demonstrated that the source of energy in the diet could affect the plasma amino acid pattern when the amount of protein ingested is kept constant (26). They also reported that when the subjects were receiving a high-fat diet (235 g of fat and 40 g of carbohydrate per day) gluconeogenesis from amino acids was increased, the concentrations of branched-chain amino acids were elevated significantly and the alanine value decreased slightly (26). However, in this study the energy percentages of fat and carbohydrate were widely different from those of the study of Swendseid et al. (26). We cannot explain clearly the reason for the differences in serum amino acids between the habitual and the low-protein diet. Further studies are needed to define the effect of dietary fat and fiber on serum amino acids.

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REFERENCES

1) Tanaka, N., Kubo, K., Shiraki, K., Koishi, H., and Yoshimura, H. (1980): A pilot study on protein metabolism in the Papua New Guinea highlanders. J. Nutr. Sci. Vitaminol., 26, 247–259.

2) Okuda, T., Kajiwaru, N., Date, C., Sugimoto, K., Rikimaru, T., Fujita, Y., and Koishi, H. (1981): Nutritional status of Papua New Guinea highlanders. J. Nutr. Sci. Vitaminol., 27, 319–331.

3) Itoh, S., Katayama, Y. S., Koishi, H., and Izumi, S. (1982): Serum concentration of protein, triglyceride, β-lipoprotein and cholesterol in Papua New Guinea highlanders. J. Nutr. Sci. Vitaminol., 28, 411–417.

4) Fujita, Y., Rikimaru, T., Okuda, T., Date, C., Kajiwaru, N., Yanase, K., and Koishi, H. (1982): Studies on protein nutrition of Papua New Guinea highlanders: nitrogen balance and hematological studies. J. Nutr. Sci. Vitaminol., 28, 431–440.

5) Oomen, H. A. P. C. (1970): Interrelationship of the human intestinal flora and protein utilization. Proc. Nutr. Soc., 29, 197–206.

6) Sinnett, P. F. (1975): The People of Murapin. Classey, E. W. Ltd., Faringdonoxon.
7) Kajiwara, N. M., Okuda, T., Miyatani, S., Date, C. Minamide, T., Fujita, Y., Ichikawa, M., Baba, M., Heywood, P., and Koishi, H. (1984): Nutritional status of Papua New Guinea highlanders: Seasonal comparison of festival and non-festival times. *J. Food Nutr.*, 41, 55–61.

8) McGovern, J. J., Jones, A. R., and Steinberg, A. G. (1955): The hematocrit of capillary blood. *New Engl. J. Med.*, 253, 308–312.

9) International Committee for Standardization in Haematology. (1967): Recommendations for haemoglobinometry in human blood. *Br. J. Haematol.* (suppl.), 13, 71–75.

10) Gibson, J. G., and Evans, W. A. (1937): Clinical studies of the blood volume. 1. Clinical application of a method employing the azo dye "Evans blue" and the spectrophotometer. *J. Clin. Invest.*, 16, 301–316.

11) Gornall, A. G., Bardawill, C. J., and David, M. M. (1949): Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.*, 177, 751–766.

12) Doumas, B. T., Watson, W. A., and Biggs, H. G. (1971): Albumin standards and the measurement of serum albumin with bromcresol green. *Clin. Chim. Acta*, 31, 87–96.

13) Kaplan, A. (1970): The determination of urea, ammonia and urease. *Methods Biochem. Anal.*, 17, 311–324.

14) Stein, W. H., and Moore, S. (1954): The free amino acids of human blood plasma. *J. Biol. Chem.*, 211, 915–926.

15) Curtain, C. C., Gajdusek, D. C., Kidson, C., Gorman, J., Champness, L., and Rodrigue, R. (1965): A study of the serum proteins of the peoples of Papua and New Guinea. *Am. J. Trop. Med. Hyg.*, 14, 678–690.

16) Eggum, B. O. (1970): Blood urea measurement as a technique for assessing protein quality. *Br. J. Nutr.*, 24, 983–988.

17) Fujita, Y., Yoshimura, Y., and Inoue, G. (1978): Effect of low-protein diets on free amino acids in plasma of young men: Effect of protein quality with maintenance of excess energy intake. *J. Nutr. Sci. Vitaminol.*, 24, 297–309.

18) Altman, P. L., and Dittmer, D. S. (eds.) (1974): Biology Data Book, 2nd Ed., Vol. 3, Federation of American Societies for Experimental Biology, Bethesda, pp. 1806–1807.

19) Swendseid, M. E., Tuttle, S. G., Figueroa, W. S., Mulcare, D., Clark, A. J., and Massey, F. J. (1966): Plasma amino acid levels of men fed diet differing in protein content. Some observations with valine-deficient diets. *J. Nutr.*, 88, 239–248.

20) Waterlow, J. C. (1969): The assessment of protein nutrition and metabolism in the whole animal, with special reference to man, in Mammalian Protein Metabolism, Vol. 3, ed. by Munro, H. N., Academic Press, New York and London, pp. 325–390.

21) Felig, P. (1973): The glucose-alanine cycle. *Metabolism*, 22, 179–207.

22) Whitehead, R. G. (1964): Rapid determination of some plasma amino acids in subclinical kwashiorkor. *Lancet*, 1, 250–252.

23) Arroyave, G. (1970): Comparative sensitivity of specific amino acid ratios versus "essential to nonessential" amino acid ratio. *Am. J. Clin. Nutr.*, 23, 703–706.

24) Smith, S. R., Pozefsky, T., and Chhetri, M. K. (1974): Nitrogen and amino acid metabolism in adults with protein-calorie malnutrition. *Metabolism*, 23, 603–618.

25) Anderson, H. L., Benevenga, N. J., and Harper, A. E. (1968): Associations among food and protein intake, serine dehydratase, and plasma amino acids. *Am. J. Physiol.*, 214, 1008–1013.

26) Swendseid, W. E., Yamada, C., Vinyard, E., Figueroa, W. G., and Drenick, E. J. (1967): Plasma amino acid levels in subjects fed isonitrogenous diets containing different proportions of fat and carbohydrate. *Am. J. Clin. Nutr.*, 20, 52–55.