EFFECT OF LOW-PROTEIN DIETS ON FREE AMINO ACIDS IN PLASMA OF YOUNG MEN:
EFFECT OF PROTEIN QUALITY WITH MAINTENANCE OR EXCESS ENERGY INTAKE

Yoshiaki Fujita, Yukio Yoshimura, and Goro Inoue

Department of Nutrition, School of Medicine, Tokushima University,
3-chome, Karamoto-cho, Tokushima 770, Japan
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Summary  Hematological changes due to protein deprivation were
studied in 34 young Japanese men who were given a standard diet with an N
intake of 200 mg/kg for one week and then low-protein diets with
maintenance (45 ± 2 kcal/kg) or excess (57 ± 2 kcal/kg) energy and N
intakes of about 44 to 99 mg/kg of whole egg or about 50 to 121 mg/kg of
polished rice for three weeks. In the period of the low-protein diet the
centrations of most individual free plasma essential amino acids (EAA)
decreased significantly in general, and thus the total FAA concentration
decreased significantly. Lowering of the valine concentration and elevation
of the alanine concentration were the highest changes. The total non-
essential amino acid (NEAA) concentration increased significantly in men
fed a low-rice-protein diet, but not in those fed a low-egg-protein diet.
Consequently, in the former group the ratio of essential to nonessential
amino acids fell significantly from 0.96 in the control period to 0.61–0.74 in
the period of consuming the low-protein diet. The effect of protein
depprivation on the plasma EAA concentration was also larger with egg
protein than with rice protein, and the total EAA concentration of men fed
egg protein changed significantly and in parallel with the N intake over the
range of 44 to 99 mg/kg. The decreases in serine and threonine and increase
in alanine tended to be more when energy intake was over the maintenance
level. The concentration of plasma proteins and especially albumin
decreased significantly during the period of consuming the low-protein diet.
The interrelation of plasma-free amino acids, the amino acid pool in tissues
and dietary N and energy intakes is briefly discussed.

Hematological changes in protein deficiencies have been studied extensively in
men and animals. Yoshimura (1) showed that measurements of the concentrations
and total amounts of plasma protein and hemoglobin were useful methods for
detecting protein deficiency in men. Whitehead (2) found a characteristic change in

1 藤田美明，吉村幸雄，井上五郎
the pattern of plasma-free amino acids in infants with PEM and proposed a "nonessential to essential amino acid ratio" as a criterion for diagnosis of this condition. Abnormal amino acid ratios have also been observed by others in human infants (3, 4) and adults (5) and in rats (6). There is also evidence that plasma-free amino acids are influenced by not only protein deficiency but also energy intake and other factors. GRIMBLE and WHITEHEAD (7) examined the effect of glucose administration on plasma-free amino acids in kwashiorkor. LUNN et al. (8) showed that the plasma amino acid pattern was affected by the changes in hormone balance that resulted from decrease in food intake.

This paper reports studies on the effect of low-protein diets of different qualities supplying maintenance or excess energy on the concentrations of plasma-free amino acids and other blood constituents in young men.

EXPERIMENTAL

The subjects tested were 34 healthy male university students of 20 to 27 years old. They were given a standard diet containing about 200 mg N/kg of mixed protein for

| Ingredient               | Amount (g) | Approx. energy (kcal) | N content (g) |
|--------------------------|------------|-----------------------|---------------|
| Rice (polished)          | 352        | 1,232                 | 4.25          |
| Whole egg                | 92         | 143                   | 1.81          |
| Tuna flake               | 37         | 57                    | 1.31          |
| Chicken                  | 37         | 50                    | 1.25          |
| Cheese                   | 12         | 44                    | 0.50          |
| Frozen bean curd         | 7          | 33                    | 0.61          |
| Pickles                  | 51         | 17                    | 0.14          |
| Cabbage                  | 102        | 25                    | 0.26          |
| Onion                    | 51         | 20                    | 0.10          |
| Carrot                   | 15         | 8                     | 0.03          |
| Cucumber                 | 51         | 5                     | 0.06          |
| Apple                    | 102        | 79                    | 0.06          |
| Potato                   | 102        | 79                    | 0.30          |
| Spinach                  | 82         | 23                    | 0.40          |
| Mayonnaise               | 20         | 99                    | 0.11          |
| Soy sauce                | 41         | 16                    | 0.46          |
| Sugar                    | 20         | 81                    |               |
| Corn oil                 | 10         | 88                    |               |
| NaCl                     | 10         |                       |               |
| Vitamins                 | 1          |                       |               |
| Minerals                 | 2          |                       |               |
| Cornstarch               | 80         | 274                   | 0.02          |
| Sugar                    | 34         | 131                   |               |
| Corn oil                 | 10         | 88                    |               |
| **Total**                | **1,318**  | **2,595**             | **11.67**     |

* The subject tested weighed 57.5 kg and received 203 mg N/kg and 45 kcal/kg. * Measured by the Kjeldahl method. * For details, see Reference (9).
one week and then a low-protein diet for three weeks; the latter containing either 44.3
98.6 mg N/kg of whole egg protein or 49.6 to 121.4 mg N/kg of polished rice
protein, and either maintenance (45 ± 2 kcal/kg) or excess (57 ± 2 kcal/kg) energy.
The composition of one of the standard diets is shown in Table 1 and the
compositions of the low-protein diets, together with details of the subjects and
experimental design, were given in the previous report (9).

Hematological tests were carried out at the end of the preliminary period and
once a week in the standard period. Blood was withdrawn from the antecubital vein
at about 7:00 a.m., before breakfast, for measurements of free amino acids,
hemoglobin, plasma protein and albumin, the hematocrit and the circulating plasma
volume. Plasma specimens for amino acid analysis were prepared by the procedure of
STEIN and MOORE (10) with a slight modification (11), and analyzed in an automatic
amino acid analyzer.2 The levels of Thr, Val, Cys, Met, Ile, Leu, Tyr, Phe, and Lys as
essential amino acids (EAAs) and Asp, Ser, Glu, Gly, Ala, His, and Arg as
nonessential amino acids (NEAAs) were measured. Plasma protein was calculated to
be 6.55 times the difference between the total and non-protein nitrogen levels,
determined by the Kjeldahl method. Plasma albumin was determined colorimetrically (12). Hemoglobin was measured by the cyanmethemoglobin method (13) and the hematocrit by high-speed centrifugation in a capillary (14). The
circulating plasma volume was determined by the dilution method with Congo
red (15) and the circulating blood volume was calculated using the hematocrit value.
The total amounts of plasma protein and hemoglobin were also calculated.

RESULTS

Changes of the free amino acid concentrations in the plasma of subjects given low-
protein diets

The mean plasma amino acid concentrations after 3 weeks on a low-egg-, or rice-
protein diet are compared with those in the preliminary period in Table 2. The mean
values for all 34 subjects at the end of the preliminary and experimental periods are
also shown graphically in Fig. 1.

The control aminogram obtained in the standard period is comparable with
those reported by others (16–18). Most of the EAAs decreased significantly in the
experimental period, the reductions in valine, leucine, and lysine being the greatest
with both proteins. The total amount of EAAs was 16% less than the control with
low-rice-protein and 23% less than the control with low-egg-protein. Among the
NEAAs, alanine increased significantly with both diets and glycine increased
significantly with rice protein but not with egg protein. Unlike in rats (19), in human
subjects serine was not affected with a maintenance energy intake and tended to
decrease with excess energy intake. Thus with the low-rice-protein diet the total

2 Yanagimoto Mfg. Co., Ltd., Yanaco, Model LC-5S.
Table 2. Effect of low-protein diets with maintenance and excess energy on free amino acid concentrations in plasma of young men. (μmoles/liter)

|                          | Standard diet | Low-rice-protein diet | Low-egg-protein diet |
|--------------------------|---------------|-----------------------|----------------------|
|                          |               | Maintenance          | Excess               | Combined          |
|                          |               | energy               | energy               |                   |
| (34)b                    |               | (14)                 | (7)                  | (21)              |
| Threonine                | 176 ± 38      | 165 ± 56             | 135 ± 27**           | 149 ± 40          |
| Valine                   | 293 ± 38      | 246 ± 54**           | 235 ± 41***          | 242 ± 49***       |
| Cystine                  | 79 ± 31       | 81 ± 35              | 90 ± 27              | 84 ± 32           |
| Methionine               | 30 ± 12       | 29 ± 11              | 25 ± 13              | 28 ± 12           |
| Isoleucine               | 87 ± 15       | 89 ± 18              | 85 ± 13              | 82 ± 16           |
| Leucine                  | 162 ± 21      | 137 ± 20***          | 139 ± 22*            | 137 ± 20***       |
| Tyrosine                 | 74 ± 16       | 62 ± 11*             | 58 ± 12*             | 61 ± 11**         |
| Phenylalanine            | 79 ± 23       | 63 ± 9*              | 58 ± 9*              | 61 ± 9**          |
| Lysine                   | 218 ± 35      | 176 ± 28***          | 174 ± 28**           | 175 ± 27***       |
| Total EAA                | 1,208 ± 150   | 1,029 ± 165***       | 998 ± 143**          | 1,019 ± 155***    |
| Aspartate                | 26 ± 14       | 26 ± 17              | 31 ± 8               | 27 ± 15           |
| Serine                   | 209 ± 35      | 217 ± 82             | 177 ± 52*            | 203 ± 74          |
| Glutamate                | 70 ± 27       | 80 ± 41              | 85 ± 22              | 82 ± 35           |
| Glycine                  | 322 ± 90      | 432 ± 167**          | 436 ± 78**           | 435 ± 144***      |
| Alanine                  | 460 ± 120     | 626 ± 190***         | 766 ± 176***         | 668 ± 193***      |
| Histidine                | 96 ± 18       | 82 ± 21*             | 82 ± 14              | 82 ± 19**         |
| Arginine                 | 93 ± 21       | 90 ± 19              | 82 ± 30              | 87 ± 13           |
| Total NEAA               | 1,278 ± 212   | 1,554 ± 325**        | 1,636 ± 210***       | 1,581 ± 289***    |
| Grand total              | 2,486 ± 318   | 2,583 ± 437          | 2,644 ± 309          | 2,600 ± 395       |
| E/N ratiof               | 0.96 ± 0.13   | 0.67 ± 0.10***       | 0.61 ± 0.08***       | 0.65 ± 0.10***    |

a Nitrogen intakes ranged from 49.6 to 121.4 and 51.0 to 88.6 mg/kg with maintenance and excess energy intakes, respectively, of rice protein and 49.6 to 98.6 and 44.3 to 79.4 mg/kg, respectively, with those of egg protein. b Figures in parentheses indicate numbers of subjects. c Represents the total numbers of subjects for maintenance and excess energy intakes. d Values represent the means ± S.D. after 1 week on standard diet and 3 weeks on a low-protein diet. e *, ** and ***: Significantly different from the value in the standard period at p<0.05, p<0.01 and p<0.001, respectively. Asterisks in parentheses indicate a significant difference between the maintenance and excess energy groups. f E/N ratio means the ratio of total EAA to total NEAA.
amount of NEAAs was significantly higher than with the control diet, whereas with the low-egg-protein diet it was not; moreover, the ratio of EAA to NEAA (E/N ratio) fell significantly from a mean of 0.96 in the control period to 0.65 with low-rice-protein and to 0.74 with low-egg-protein diet. As seen in Fig. 2, the total EAA concentration and E/N ratio decreased markedly at the first week and then remained at lower levels.

With both proteins, fall in threonine and rise in alanine were more marked with excess energy intake than with maintenance energy intake (Table 2). With egg protein, the total EAA concentration was significantly lower with excess energy than with maintenance energy. With each protein, there was no significant difference between the total NEAA concentrations in the groups with excess and maintenance energy intakes because increase in alanine occurred concomitantly with decrease in serine concentration in the groups with excess energy intake.
Correlation between the free amino acid concentration in the plasma and protein intakes in the submaintenance range

The correlations between the plasma levels of free amino acids and dietary N intake in the range of about 44 to 121 mg/kg were tested statistically (Table 3). Significant correlations were observed only for the 34 subjects for the rise in alanine

![Figure 2: Time course of changes during the low-protein period in the total essential amino acid concentration and the E/N ratio. The amino acids measured were Thr, Val, Cys, Met, Ile, Leu, Tyr, Phe, and Lys as EAA and Asp, Ser, Glu, Gly, Ala, His, and Arg as NEAA. ⧫ and ⧫ represent means ± S.D. for the low-egg-protein diet (n:13) and the low-rice-protein diet (n:21), respectively. * and **: Significantly different from the standard value (⧫) at p<0.05 and p<0.01, respectively.]

![Figure 3: Relationship between dietary N intake and plasma total FAA in young men with N intakes of 44 to 121 mg/kg. The mean values for total EAA after 3 weeks on low-protein diet are plotted against the nitrogen intake. ⧫ represents the means ± S.D. for the 34 subjects in the control period. The regression equations calculated are as follows: with egg protein (.–.–), Y = 3.67X + 676.9 (n:13, r=0.702, p<0.01); with rice protein (○○), Y = −1.56X + 1,148.1 (n:21, r=−0.249, NS), where X is the N intake (mg/kg) and Y is the total EAA (μmoles/liter).]
Table 3. Correlation coefficients between dietary nitrogen intakes and individual concentrations of free amino acids in the plasma of young men fed low-protein diets.

| Low-protein diet | Maintenance energy (7) | Excess energy (8) | Combined energy (9) | Excess NEAA (10) | Combined NEAA (11) | Correlation coefficients |
|------------------|------------------------|-------------------|---------------------|----------------|--------------------|------------------------|
| Threonine        | 0.490**                | -0.278            | -0.278              | 0.249          | 0.249              | 0.249                  |
| Valine           | 0.088                  | 0.140             | 0.140               | 0.140          | 0.140              | 0.140                  |
| Cystine          | 0.569*                 | -0.388            | -0.388              | -0.388         | -0.388             | -0.388                 |
| Methionine       | 0.436                  | -0.337            | -0.337              | -0.337         | -0.337             | -0.337                 |
| Isoleucine       | 0.189                  | 0.096             | 0.096               | 0.096          | 0.096              | 0.096                  |
| Leucine          | 0.437                  | 0.317             | 0.317               | 0.317          | 0.317              | 0.317                  |
| Tyrosine         | 0.501                  | 0.276             | 0.276               | 0.276          | 0.276              | 0.276                  |
| Phenylalanine    | 0.347                  | -0.153            | -0.153              | -0.153         | -0.153             | -0.153                 |
| Lysine           | -0.442                 | 0.154             | 0.154               | 0.154          | 0.154              | 0.154                  |
| Aspartate        | 0.056                  | 0.062             | 0.062               | 0.062          | 0.062              | 0.062                  |
| Serine           | 0.765**                | -0.212            | -0.212              | -0.212         | -0.212             | -0.212                 |
| Glutamine        | 0.312**                | 0.061             | 0.061               | 0.061          | 0.061              | 0.061                  |
| Glutamate        | 0.996                  | -0.344            | -0.344              | -0.344         | -0.344             | -0.344                 |
| Proline          | 0.748**                | -0.574**          | -0.574**            | -0.574**       | -0.574**           | -0.574**               |
| Total EAA        | 0.642                  | -0.249            | -0.249              | -0.249         | -0.249             | -0.249                 |
| Total NEAA       | 0.369                  | -0.357            | -0.357              | -0.357         | -0.357             | -0.357                 |
| Total N          | 0.415                  | -0.343            | -0.343              | -0.343         | -0.343             | -0.343                 |
| E/N ratio        | 0.074                  | 0.157             | 0.157               | 0.157          | 0.157              | 0.157                  |

** Figures are correlation coefficients calculated on values after 3 weeks of the low-protein diets. * and **: Represent that the correlation coefficients are statistically significant at p<0.05 and p<0.01, respectively. Other explanations are as for Table 2.
and fall in serine (Table 3, last column). In general, with egg protein the correlation coefficients for individual amino acids were estimated to be comparably high, while with rice protein they were considerably lower and in many cases were inverse. Alanine and histidine in rice and valine in egg were relatively sensitive to protein deficiency, and thus there was a significant correlation between total EAA and N intake with egg protein. This correlation is shown graphically in Fig. 3. No significant correlation was observed with rice protein.

**Changes in hemoglobin, plasma protein and other characters**

Other hematological data are summarized in Table 4. A low-protein diet for 3 weeks did not decrease the concentration or total amount of hemoglobin significantly, but decreased the plasma protein, affecting the concentration more than the total amount. The mean albumin concentration in 13 subjects decreased significantly, being 4.54 g/100 ml in the control period and 4.09 g/100 ml after one week of the experimental period (Fig. 4). No change was observed in the hematocrit or circulating plasma volume and supply of excess energy did not affect the values either.

![Fig. 4. Time course of changes in plasma albumin concentration measured in 13 subjects given low-egg-protein diets with 44 to 99 mg N/kg for 3 weeks. **: Significantly different from the value for the control period at p < 0.01. ‡: means ± S.D.](image)

**DISCUSSION**

As shown here and by others (2–6), the fall in the E/N ratio in the plasma of subjects given low-protein diets was mainly caused by decrease in the concentration of total EAA, and especially branched-chain amino acids, and partly by increase in the concentration of alanine. The decrease in concentration of plasma EAA was primarily due to reduced supply of these amino acids but it was also related to energy supply, because addition of extra energy to the low-egg-protein diet resulted in reduction of plasma EAAs. Further evidence that decrease in the levels of plasma EAAs in protein deficiencies may be intensified by supply of excess energy is as
Table 4. Changes in blood properties of young men given low-protein diets with maintenance and excess energy.

|                      | Standard diet (34) | Maintenance energy (14) | Excess energy (7) | Combined (21) | Maintenance energy (7) | Excess energy (6) | Combined (13) |
|----------------------|--------------------|-------------------------|-------------------|---------------|-------------------------|-------------------|---------------|
| **Hemoglobin**       |                    |                         |                   |               |                         |                   |               |
| Concentration        | 15.5 ± 1.5c        | 14.7 ± 2.0c             | 15.1 ± 1.3        | 14.8 ± 1.7    | 14.8 ± 1.1              | 15.2 ± 0.6        | 15.0 ± 0.9    |
| Total amount         | 14.1 ± 1.7d        | 12.7 ± 2.1c**           | 14.2 ± 1.7        | 13.3 ± 2.0f   | 13.3 ± 1.1              | 13.5 ± 1.1        | 13.4 ± 1.1    |
| **Plasma protein**   |                    |                         |                   |               |                         |                   |               |
| Concentration        | 7.06 ± 0.39        | 6.77 ± 0.42*            | 6.57 ± 0.38**     | 6.69 ± 0.40** | 6.67 ± 0.35*            | 6.78 ± 0.36       | 6.71 ± 0.35** |
| Total amount         | 3.74 ± 0.47d       | 3.37 ± 0.43**           | 3.54 ± 0.29       | 3.44 ± 0.34*  | 3.47 ± 0.34             | 3.31 ± 0.20*      | 3.41 ± 0.30*  |
| **Hematocrit**       | 42.6 ± 2.8         | 40.8 ± 2.6*             | 41.6 ± 4.0        | 41.1 ± 3.1    | 40.9 ± 3.8              | 45.6 ± 1.7*       | 42.5 ± 3.9    |
| **Circulating**      |                    |                         |                   |               |                         |                   |               |
| Plasma volume        | 53.3 ± 6.5d        | 48.2 ± 6.2**            | 55.3 ± 3.9        | 52.3 ± 5.5f   | 52.1 ± 4.8              | 48.2 ± 3.7        | 50.7 ± 4.7    |
| Blood volume         | 92.4 ± 9.8d        | 84.3 ± 6.0**            | 93.6 ± 8.6        | 87.6 ± 8.1f   | 88.5 ± 6.7              | 88.5 ± 7.6        | 88.5 ± 6.8    |

* Figures in parentheses are numbers of subjects. b Concentration and total amount represent gram per 100 ml of blood volume and gram per kg of body weight, respectively. c Values are means ± S.D., after one week on standard diet and 3 weeks on low-protein diet. d,e and f: Values for 30, 10 and 17 subjects, respectively. * and **: Significantly different from the value in the period on standard diet at p < 0.05 and p < 0.01, respectively. h Circulating blood volume was calculated from the values for the circulating plasma volume and hematocrit.
follows: (i) Lunn et al. (8) observed hyperinsulinemia resulting from ingestion of high-carbohydrate meals during the early stage of kwashiorkor and suggested that utilization of amino acids in tissues was enhanced in this condition; (ii) We found previously (9) that the nitrogen balance and NPU observed with submaintenance N intakes were greatly affected by the protein sparing effect of energy intake; (iii) Munro et al. (20) showed that the lowered levels of plasma amino acids observed in subjects loaded with glucose were due to enhanced utilization of the amino acids in the muscle through the action of insulin; and (iv) recently, Grimble and Whitehead (7) also reported similar findings to (iii), in patients with kwashiorkor after administration of glucose.

In this work the levels of branched-chain amino acids decreased most during protein deficiency, and this is consistent with findings in cases of kwashiorkor (21). The threonine concentration was significantly lowered in our subjects receiving excess energy; however, it was reported to increase in obese subjects during starvation (22). This discrepancy needs further study.

Lunn et al. (8) observed that the plasma alanine concentration in infants with kwashiorkor was increased by high-carbohydrate food. Moreover, Adibi (17) observed a high concentration of plasma alanine in adults fed a protein-free diet, but a low concentration in starved subjects. Furthermore, Grimble and Whitehead (23) reported high alanine levels in children fed diets low in protein but adequate in energy. We found that elevation of plasma alanine was intensified by supply of excess energy (Table 2). Supply of energy probably increases the alanine concentration because it reduces gluconeogenesis, for which alanine is a major substrate (24).

In contrast to plasma alanine, plasma serine was normal or slightly reduced in protein deficiency. These findings are consistent with those of Young et al. (25). On the other hand, Fallon et al. (26, 27) observed that in rats on low-protein diet, serine degrading enzyme activity decreased and serine synthetic activity in the liver increased, suggesting a decrease of plasma serine. We have also observed marked increases of plasma and liver serine in adult rats on a low-protein diet (unpublished data3). Species differences in serine metabolism in protein deficiency must be examined.

We found a significant correlation between the total EAA concentration in the plasma and N intake over the range of 44 to 99 mg/kg in subjects given a low-egg-protein diet, but not in those given a low-rice-protein diet. Young et al. (25) observed a similar correlation in young men receiving 0.2 to 0.5 g/kg of a low-egg-protein diet. However, in our subjects the E/N ratio was not significantly correlated with the protein intake, indicating that individual amino acids in the plasma were affected in complex ways by not only the protein level but also the protein source and energy content of the diet.

3 Fujita, Y., Kuge, C. and Inoue, G.: Relationship between dietary protein levels and free amino acid concentrations in the plasma and tissues of rats. Abstracts of the 24th General Meeting of the Japanese Society of Food and Nutrition, Sapporo, Sept. 2-4, 1970.
Our previous work showed that the protein sparing action of energy intake had more effect than the protein intake itself on N utilization in men with a submaintenance intake of N (unpublished data by Inoue et al.4). Singh et al. (28) observed increased levels of plasma EAAs in marasmic children and similar findings were obtained by Salem et al. (6) in protein-energy-deficient rats. These observations suggest that increase in plasma EAAs may be due to protein catabolism in skeletal muscle during energy deprivation. To elucidate the nutritional significance of change in the level of plasma amino acids in protein deficiency, the dynamic states of plasma amino acids in relation to amino acid pools in the tissues must be clarified (29, 30). The relationship between the effects of nitrogen and energy intakes on amino acid utilization must be studied.

Many years ago, in comprehensive long-term studies Yoshimura's group (1, 31–33) observed significant decreases in the concentrations and amounts of plasma proteins and hemoglobin in young men receiving a low-protein diet. In the present study, the concentration of plasma protein, and especially albumin, was also found to be a sensitive parameter of protein intake. This finding is also consistent with results in malnourished children by Duzen et al. (34) and Padilla et al. (35) and in protein-deprived dog by Heard et al. (36). As reported previously (9), the maintenance N requirements for subjects with a maintenance energy intake were about 90 mg/kg for egg protein and about 120 mg/kg for rice protein. This maintenance level of N intake corresponds to an E/N ratio of 0.71 and plasma protein concentration of 6.91 g/100 ml and these values are significantly lower than those of 0.95 and 7.30 g/100 ml, respectively, for the same subjects in the control period. This implies that an N intake sufficient to maintain a zero balance may not be sufficient to maintain hematological values at physiologically normal levels.

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