EFFECT OF LOW PROTEIN DIETS ON FREE AMINO ACIDS IN PLASMA OF YOUNG MEN: EFFECT OF WHEAT GLUTEN DIET

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Summary  Studies were made on alterations in plasma amino acids in young men fed a diet containing graded levels of wheat gluten. After one week on a standard diet containing 200 mgN/kg of mixed protein (animal protein content 45%), 38 young men were given a wheat gluten diet containing 170, 100, 60, 30, 15 or zero mgN/kg for 2 weeks. Blood samples measuring plasma free amino acids were taken before breakfast at the end of the periods on a standard diet and an experimental diet. In subjects on diets containing 170 to 30 mgN/kg the plasma concentrations of threonine, valine, methionine, leucine, tyrosine, phenylalanine, serine, histidine and arginine fell significantly with decrease in protein intake, but the concentration of alanine increased significantly. On the other hand, in subjects on diets containing 15 or zero mgN/kg, the plasma concentrations of the essential amino acids did not decrease, but increased to slightly more than in subjects on a diet containing 30 mgN/kg, and the alanine and glycine concentrations increased steadily. Values for plasma lysine varied from 146 ± 22 to 194 ± 31 μmoles/liter with gluten intakes of 170 to zero mgN/kg, but were comparable with that of 186 ± 33 μmoles/liter in subjects on a standard diet, showing that the plasma lysine concentration did not clearly reflect the dietary concentration of lysine in young men on a wheat gluten diet.

Keywords  wheat gluten, free amino acids, lysine, protein free diet, standard aminogram
When a diet deficient in an essential amino acid is given to animals, the concentration of the missing amino acid in plasma decreased (1-4). Jacobs and Crandal (5) reported that administration of wheat gluten to growing rats caused an appreciable decrease in the free lysine concentration in the plasma and inhibited growth. Graham et al. (6) showed that plasma lysine in children is greatly influenced by the dietary lysine level. As we reported previously (7), when young men were given a low egg or rice protein diet for three weeks, their plasma concentrations of lysine and other essential amino acids (EAAs) decreased significantly. Unpublished data from our laboratory, however, showed that the concentration of free lysine in the plasma and tissues did not decrease in adult rats fed diets containing different levels of wheat gluten for four weeks. Furthermore, in starving chicks (8,9) and sheep (10) and protein-deprived men (11) the plasma lysine concentration remains normal or increases. Moreover, Edwards et al. (12) showed that plasma free lysine was not affected significantly by supplying a wheat gluten diet to human adults. These conflicting results suggest that there is a difference in the responses of the plasma lysine concentration in growing and adult animals and humans to a wheat gluten diet.

The primary object in this study was to gain more detailed information on changes in the plasma amino acid concentrations, particularly lysine, in young men on diets containing various levels of wheat gluten. To clarify the results obtained with wheat gluten, we also examined plasma aminograms of subjects fed diets with adequate protein and a protein-free diet.

**EXPERIMENTAL**

The subjects were 38 healthy male university students 19 to 24 years of age. They were given a standard diet containing 200 mgN/kg of mixed protein (animal protein content 45%) for one week and then diets containing graded levels of wheat gluten or a protein-free diet for 2 weeks. The level of wheat gluten in the diet was 170 mgN/kg for 4 subjects, 100 mgN/kg for 3, 60 mgN/kg for 3, 30 mgN/kg for 10 and 15 mgN/kg for 9 subjects. Nine subjects were given a protein-free diet. Throughout the experiment the energy intakes of the subjects were maintained at 47 to 50 kcal/kg, which is sufficient to meet energy requirements. The energy intakes of the subjects on a protein-free diet were excessively maintained at about 54 kcal/kg to obtain the least level of endogenous N excretion, though the excess energy supply has little influence on N output of the subjects fed a protein-free diet (13). The compositions of one wheat gluten diet and the protein-free diet are shown in Table 1.

Plasma amino acid analysis was carried out at the end of the standard diet and at the end of the experimental period. Blood samples were withdrawn from the antecubital vein before breakfast. The procedures and methods for amino acid analysis were essentially as reported previously (7).
RESULTS

Body weight and nitrogen balance

Results of body weight and nitrogen balance of the subjects fed graded levels of wheat gluten are shown in Table 2. Throughout the experiment the energy intakes of the subjects were maintained at about 54 kcal/kg for the protein-free group and at 47 to 50 kcal/kg for the other groups, which is sufficient to meet energy requirements. However, their body weights tended to decrease in the experimental periods the amounts being -0.89, -0.28, -0.75, -0.21, -0.60 and 0.97 kg/2 weeks at N-intakes of 170, 100, 60, 30, 15 and zero mg N/kg, respectively. In addition, their nitrogen balances were negative irrespective of the dietary N levels, even in the 170 mg N/kg group.

Plasma amino acid pattern of Japanese adults on a standard diet

The plasma amino acid pattern, measured on samples taken early in the morning from healthy male university students on a standard diet, is shown in Fig. 1 and Table 3. Of the individual essential amino acids, valine had the highest concentration (mean value, 285 μmoles/liter) and lowest coefficient of variation (11%). Branched-chain amino acids constituted the highest proportion (45%) of total EAA followed in order by lysine, threonine and aromatic and sulfur-containing amino acids. Of the nonessential amino acids (NEEAs), the mean value
Table 2. Effects of graded levels of wheat gluten on nitrogen balance and body weight of young men.

| Level of Gluten (mg/kg) | N intake (mg/kg) | N balance (mg/kg) | Body weight (kg) | Experimental diet | Difference |
|------------------------|------------------|-------------------|------------------|-------------------|------------|
| Zero                   | 173.6±0.8        | 100.4±0.7         | 60.5±1.8         | -5.9±2.5          | -0.39±0.35|
| 15 mgN/kg              | 160.6±0.7        | 104.5±1.0         | 62.2±1.6         | 5.6±2.4           | 0.28±0.51 |
| 30 mgN/kg              | 158.6±0.3        | 106.1±1.0         | 63.0±1.0         | 7.6±2.4           | 0.21±0.21 |
| 60 mgN/kg              | 156.6±0.3        | 106.0±1.0         | 63.0±1.0         | 7.6±2.4           | 0.21±0.21 |
| 150 mgN/kg             | 154.6±0.3        | 106.0±1.0         | 63.0±1.0         | 7.6±2.4           | 0.21±0.21 |

*Figures in parentheses indicate numbers of subjects.

Significantly different from the value in the standard diet at p<0.05, p<0.01 and p<0.001, respectively.

The subjects were given a standard diet for one week and then graded levels of wheat gluten for 2 weeks. Values are means ± SD.

Mean values of urinary N for the last 5 days and of fecal N for the entire period in the experimental period were used for estimating the nitrogen balance.

* Values represent means ± SD of the last 5 days.
Table 3. Effects of graded levels of wheat gluten on free amino acid concentrations in plasma of young men. (µmoles/liter)

|                    | Standard diet (68) | 170 mgN/kg (4) | 100 mgN/kg (3) | 60 mgN/kg (3) | 30 mgN/kg (10) | 15 mgN/kg (9) | Zero (9) |
|--------------------|--------------------|----------------|----------------|----------------|----------------|----------------|----------|
| Threonine          | 168 ± 38<sup>a</sup> | 169 ± 25<sup>b</sup> | 114 ± 14<sup>**</sup> | 129 ± 15 | 108 ± 24<sup>***</sup> | 115 ± 23<sup>***</sup> | 149 ± 26 |
| Valine             | 285 ± 32<sup>a</sup> | 235 ± 16<sup>**</sup> | 178 ± 28<sup>***</sup> | 182 ± 21<sup>***</sup> | 145 ± 18<sup>***</sup> | 150 ± 24<sup>***</sup> | 200 ± 30<sup>***</sup> |
| Cystine            | 81 ± 28<sup>a</sup> | 60 ± 12<sup>a</sup> | 119 ± 10<sup>**</sup> | 94 ± 17<sup>***</sup> | 73 ± 31<sup>***</sup> | 75 ± 20<sup>***</sup> | 42 ± 9<sup>***</sup> |
| Methionine         | 23 ± 13<sup>a</sup> | 31 ± 4<sup>a</sup> | 14 ± 7<sup>a</sup> | 19 ± 11<sup>***</sup> | 12 ± 9<sup>***</sup> | 9 ± 9<sup>***</sup> | 27 ± 6<sup>***</sup> |
| Isoleucine         | 86 ± 14<sup>a</sup> | 73 ± 8<sup>a</sup> | 84 ± 6<sup>a</sup> | 81 ± 13<sup>***</sup> | 61 ± 12<sup>***</sup> | 56 ± 7<sup>***</sup> | 65 ± 9<sup>***</sup> |
| Leucine            | 157 ± 21<sup>a</sup> | 136 ± 9<sup>a</sup> | 149 ± 12<sup>a</sup> | 133 ± 23<sup>***</sup> | 108 ± 15<sup>***</sup> | 106 ± 7<sup>***</sup> | 126 ± 31<sup>***</sup> |
| Tyrosine           | 69 ± 15<sup>a</sup> | 63 ± 8<sup>a</sup> | 58 ± 11<sup>a</sup> | 51 ± 2<sup>***</sup> | 48 ± 9<sup>***</sup> | 55 ± 11<sup>***</sup> | 61 ± 11<sup>***</sup> |
| Phenylalanine      | 71 ± 20<sup>a</sup> | 72 ± 12<sup>a</sup> | 64 ± 23<sup>a</sup> | 44 ± 7<sup>***</sup> | 46 ± 9<sup>***</sup> | 53 ± 7<sup>***</sup> | 57 ± 9<sup>***</sup> |
| Lysine             | 208 ± 38<sup>a</sup> | 146 ± 22<sup>**</sup> | 195 ± 6<sup>a</sup> | 177 ± 8<sup>***</sup> | 157 ± 9<sup>***</sup> | 182 ± 47<sup>a</sup> | 194 ± 31 |
| **Total EAA**      | 1,152 ± 137<sup>a</sup> | 984 ± 46<sup>**</sup> | 971 ± 80<sup>a</sup> | 904 ± 68<sup>***</sup> | 770 ± 69<sup>***</sup> | 805 ± 35<sup>***</sup> | 921 ± 125<sup>***</sup> |
| Aspartate          | 26 ± 11           | 11 ± 2<sup>**</sup>  | 18 ± 2           | 37 ± 8           | 17 ± 7<sup>***</sup> | 29 ± 13           | 28 ± 13           |
| Serine             | 193 ± 37<sup>a</sup> | 350 ± 68<sup>***</sup> | 192 ± 15<sup>a</sup> | 159 ± 11| 148 ± 18<sup>***</sup> | 165 ± 52<sup>a</sup> | 205 ± 35 |
| Glutamate          | 89 ± 51<sup>a</sup> | 66 ± 22<sup>a</sup> | 204 ± 32<sup>***</sup> | 164 ± 53<sup>a</sup> | 114 ± 32<sup>***</sup> | 147 ± 92<sup>***</sup> | 58 ± 12<sup>***</sup> |
| Glycine            | 286 ± 80<sup>a</sup> | 314 ± 41<sup>a</sup> | 328 ± 54<sup>a</sup> | 291 ± 134<sup>a</sup> | 292 ± 77<sup>***</sup> | 340 ± 36<sup>a</sup> | 380 ± 83<sup>***</sup> |
| Alanine            | 445 ± 99<sup>a</sup> | 504 ± 48<sup>a</sup> | 465 ± 65<sup>a</sup> | 513 ± 181<sup>a</sup> | 663 ± 147<sup>***</sup> | 655 ± 118<sup>***</sup> | 768 ± 87<sup>***</sup> |
| Histidine          | 95 ± 15<sup>a</sup> | 92 ± 11<sup>a</sup> | 101 ± 9<sup>a</sup> | 87 ± 7<sup>***</sup> | 76 ± 13<sup>***</sup> | 89 ± 21<sup>a</sup> | 81 ± 14<sup>***</sup> |
| Arginine           | 86 ± 21           | 88 ± 9           | 84 ± 19           | 95 ± 10           | 61 ± 8<sup>***</sup> | 69 ± 9<sup>***</sup> | 73 ± 18           |
| **Total NEAA**     | 1,222 ± 177<sup>a</sup> | 1,424 ± 62<sup>**</sup> | 1,392 ± 63<sup>a</sup> | 1,305 ± 434<sup>a</sup> | 1,316 ± 124<sup>***</sup> | 1,454 ± 147<sup>***</sup> | 1,593 ± 196<sup>***</sup> |
| Grand total        | 2,374 ± 277<sup>a</sup> | 2,408 ± 84<sup>a</sup> | 2,362 ± 122<sup>a</sup> | 2,209 ± 501<sup>a</sup> | 2,075 ± 168<sup>***</sup> | 2,260 ± 163<sup>***</sup> | 2,515 ± 273 |
| E/N ratio<sup>d</sup> | 0.95 ± 0.11<sup>a</sup> | 0.69 ± 0.04<sup>***</sup> | 0.70 ± 0.05<sup>***</sup> | 0.74 ± 0.22<sup>***</sup> | 0.59 ± 0.05<sup>***</sup> | 0.55 ± 0.05<sup>***</sup> | 0.58 ± 0.08<sup>***</sup> |

<sup>a</sup> Figures in parentheses indicate numbers of subjects. Four results for the 170 mgN/kg group in the standard were omitted because their N intakes were slightly low, and the value for 68 subjects in the standard diet is from 34 subjects in the present study with 34 in a previous study (7).

<sup>b</sup> Values represent means ± SD, after one week on a standard diet and 2 weeks on gluten diets.

<sup>c</sup>, <sup>***</sup> and <sup>****</sup> 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 significant differences from the values for the zero group.

<sup>d</sup> E/N ratio means the ratio of total EAA to total NEAA.
Fig. 1. Standard plasma aminogram of young Japanese men. The subjects were given basal diets containing 200 mgN/kg of mixed protein (animal protein, 45%) with a maintenance level of energy for one week. Values are means ± SD (vertical bar) for 68 subjects: 34 in the present study with 34 in a previous study (7). Aro and Sul represent phenylalanine plus tyrosine and methionine plus cystine, respectively.

Plasma amino acid pattern in subjects on a protein-free diet

The plasma amino acid pattern of subjects on a protein-free diet is shown in Table 4, with that for subjects on a standard diet for comparison. Characteristic changes appeared within one week and these alterations were then maintained. Findings were as follows: branched-chain amino acids, especially valine, decreased by about 25%. Alanine and glycine increased markedly by 73% and 37%, respectively, by the end of the 2-week experimental period. Lysine was maintained at nearly the standard level. As a result, after two weeks the value for total EAA had decreased by about 18% and that for total NEAA had increased by about 39%.
### Table 4. Changes in free amino acid concentrations in plasma of young men fed a protein-free diet. (µmoles/liter)

|                     | Standard diet | Protein-free diet | Protein-free diet |
|---------------------|---------------|-------------------|-------------------|
|                     | Day 7         | Day 14            |                   |
| Threonine           | 173±37        | 146±38            | 149±26            |
| Valine              | 287±20        | 190±33            | 200±30            |
| Cystine             | 54±19         | 41±9              | 42±9              |
| Methionine          | 25±4          | 30±6              | 27±6              |
| Isoleucine          | 81±10         | 68±10             | 65±9              |
| Leucine             | 145±19        | 118±18            | 126±31            |
| Tyrosine            | 70±14         | 59±8              | 61±11             |
| Phenylalanine       | 73±17         | 57±9              | 57±9              |
| Lysine              | 210±33        | 202±34            | 194±31            |
| **Total EAA**       | 1,117±82      | 910±113           | 921±125           |
| Aspartate           | 21±9          | 24±13             | 28±13             |
| Serine              | 188±36        | 185±32            | 205±35            |
| Glutamate           | 48±12         | 56±13             | 58±12             |
| Glycine             | 278±51        | 352±77            | 380±83            |
| Alanine             | 444±81        | 685±133           | 768±87            |
| Histidine           | 86±10         | 71±13             | 81±14             |
| Arginine            | 83±15         | 66±17             | 73±18             |
| **Total NEAA**      | 1,149±131     | 1,439±133         | 1,593±196         |
| Grand total         | 2,266±197     | 2,349±178         | 2,515±273         |
| E/N ratio           | 0.98±0.09     | 0.64±0.10         | 0.58±0.08         |

* Values are means for nine subjects ± SD, after one week on a standard diet.

** and *** Significantly different from the values in the standard period at p<0.05, p<0.01 and p<0.001, respectively.

E/N ratio means the ratio of total EAA to total NEAA.

39%, resulting in a decrease of the E/N ratio from 0.98 in the control period to 0.58 in the period on a protein-free diet.

**Effect of wheat gluten diet on the plasma amino acid pattern**

As described previously (7), the N balance was negative, even when the protein intake from wheat gluten was about 1.0 g/kg. The dietary lysine intakes were calculated to be 1.6, 3.3, 6.5, 9.8 and 18.5 mg/kg/day at N-intakes of 15, 30, 60, 100 and 170 mg/kg, respectively. Regression analysis showed that the lysine requirement for zero maintenance was 24.3 mg/kg, although from results with an amino acid mixture the safety intake was calculated as 11.4 mg/kg by Rose et al. (14) and 13.8 mg/kg by Inoue et al.²

² Inoue, G.: Studies on protein and amino acid nutrition in human adults. The memorial lecture by the Takeda Prize winner presented at the 18th General Meeting of the Japanese Society of Food and Nutrition, Nagoya, April, 1964.
As shown in Table 3, the plasma lysine concentration in subjects on a gluten diet were less than those in subjects on a standard diet. The concentration at N-intakes of 100, 60 and 15 mgN/kg did not decrease significantly, the mean values remaining at about 90% of the standard value. There was no significant correlation between lysine intake and the plasma lysine concentration, as seen in Table 5. These findings suggest that in human adults receiving a wheat gluten diet, lysine deficiency is not reflected in a decrease in the lysine concentration of the plasma.

Apart from lysine, individual EAA tended to decrease with increase in protein deficiency and the decrease in valine was greatest, as observed previously. Total EAA also decreased significantly with decrease in protein intake, being about 30% lower than the standard value at N-intakes of 30 and 15 mg/kg. Among the NEAAs, alanine increased most (about 50%) at N-intakes of less than 30 mg/kg, although this change was less with higher N-intakes. The changes in serine, glycine

| Table 5. Relation between dietary nitrogen intake and individual concentrations of free amino acids in the plasma of young men fed diets containing 30 to 170 mgN/kg wheat gluten. |
|---------------------------------------------------------------|
| Correlation coefficienta | Regression equationa |
| Threonine | 0.705**b | Y = 0.385X + 96.3 |
| Valine | 0.865** | Y = 0.586X + 131.5 |
| Cystine | -0.239 | Y = -0.098X + 92.0 |
| Methionine | 0.664** | Y = 0.115X + 8.5 |
| Isoleucine | 0.381 | Y = 0.092X + 63.1 |
| Leucine | 0.570** | Y = 0.213X + 108.1 |
| Tyrosine | 0.577** | Y = 0.101X + 45.4 |
| Phenylalanine | 0.680** | Y = 0.138X + 40.1 |
| Lysine | -0.116 | Y = -0.040X + 166.7 |
| Total EAA | 0.763** | Y = 1.509X + 755.4 |
| Aspartate | -0.413 | Y = -0.064X + 24.3 |
| Serine | 0.900** | Y = 1.322X + 101.4 |
| Glutamate | -0.226 | Y = -0.213X + 140.5 |
| Glycine | 0.147 | Y = 0.187X + 288.1 |
| Alanine | -0.451* | Y = -1.152X + 661.9 |
| Histidine | 0.511* | Y = 0.124X + 76.0 |
| Arginine | 0.573** | Y = 0.172X + 62.7 |
| Total NEAA | 0.274 | Y = 0.826X + 1,287.2 |
| Grand total | 0.555* | Y = 2.411X + 2,031.7 |
| E/N ratio | 0.407 | Y = 0.001X + 0.593 |

* Correlation coefficients and regression equations were calculated from values after 2 weeks on experimental diets in the range of 30 to 170 mgN/kg, where X is the N intake (mg/kg) and Y is the amino acid concentration (μmoles/liter).

b * and ** Significant at p < 0.05 and p < 0.01, respectively. Values were obtained for 20 subjects.
and other NEAA were not marked. Thus, a significant increase in total NEAA was observed only in subjects on diets with very low gluten contents. The mean E/N ratio ranged from 0.74 to 0.55 in the experimental period and the values were significantly lower than in the standard period.

The relationships between the plasma levels of free amino acids and the dietary N intakes for diets of 30 to 170 mgN/kg were tested statistically (Table 5). Most EAAs except lysine changed significantly in parallel with the N intake, but a significant inverse correlation was observed for alanine. A highly significant correlation was also found for serine, although it was not observed with 200 mgN/kg (standard diet). There was no significant correlation between the E/N ratio and gluten level, although the total EAA changed significantly in parallel with the N intake.

The following points are also of interest: The lowest values for total EAA and total NEAA were obtained with 30 mgN/kg, and the values increased progressively with a further decrease in protein to 15 and zero mgN/kg, as seen in Fig. 2. The values for EAA and NEAA of the protein-free group differed significantly from those for the group on 30 mgN/kg, but not from those for the group on 15 mgN/kg, due to the high variations of threonine, valine, lysine, serine, glycine

![Fig. 2. Effect of graded levels of wheat gluten on total EAA and total NEAA in the plasma of young men. The subjects were given a standard diet containing 200 mgN/kg of mixed protein for one week and then graded levels of wheat gluten for 2 weeks. Symbols represent means±SD, after one week on a standard diet and 2 weeks on a gluten diet. For numbers of subjects, see Table 3. Total EAA consists of Thr, Val, Cys, Met, Ile, Leu, Tyr, Phe and Lys and total NEAA consists of Asp, Ser, Glu, Gly, Ala, His and Arg.](image-url)
DISCUSSION

The standard aminogram for Japanese adults obtained in this study is comparable with those of WELLER et al. (11), ADIBI (15) and others (16–18) and with those for Japanese adults by OKUDA et al. (19), despite differences in the diets of the subjects examined. The A/E ratio calculated from this standard aminogram is different from that of egg proteins and from the provisional amino acid scoring pattern proposed by FAO/WHO 1973, which was estimated from human amino acid requirements (20) (Table 6).

|                | Standard plasma aminogramb | FAO/WHO pattern (1973)c | Egg proteinc |
|----------------|---------------------------|-------------------------|--------------|
| Thr            | 14.6                      | 9.0                     | 9.6          |
| Val            | 24.7                      | 12.4                    | 13.5         |
| Ile            | 7.5                       | 12.4                    | 11.0         |
| Leu            | 13.6                      | 17.2                    | 17.6         |
| Met+Cys        | 9.0                       | 16.6                    | 11.6         |
| Phe+Tyr        | 12.2                      | 17.2                    | 19.0         |
| Lys            | 18.1                      | 15.2                    | 14.3         |

a Values show amounts of individual amino acids as percentages of the total amounts of the nine amino acids indicated in the table.
b From the amino acid concentration in the plasma of young men fed standard diets containing 1.25g/kg protein for one week.
c Cited from “Energy and Protein Requirements” in the Report of the Joint FAO/WHO Ad Hoc Expert Committee (1973), published by FAO and WHO, Geneva, Switzerland.

We showed previously that in young men on a low egg- or rice-protein diet, essential amino acids in the plasma tended to decrease, while some nonessential amino acids increased (7). Similar results were also found in subjects receiving low levels of wheat gluten.

The average concentration of plasma free lysine measured in 29 subjects receiving graded levels of wheat gluten in their diet was 169 μmoles/liter, which is significantly lower than the value for the standard diet, but not significantly different from the mean value of 179 μmoles/liter reported previously for 34 subjects on diets with low contents of egg- or rice-protein (7). We found that the decrease in plasma lysine concentration with decrease in N-intake below 170mgN/kg was less than the changes in other plasma EAAs (Table 3). These
findings are consistent with the report of Young et al. (21) that the concentration of plasma lysine, unlike those of valine and other EAAs, was not correlated with a decrease in lysine intake below 7 mg/kg. We also found that a protein-free diet had very little effect on the plasma concentration of lysine, as reported by others (9,11), and observed in young men on a diet containing a lysine-free amino acid mixture (21). As is well known, when growing animals were given a gluten diet, their plasma lysine concentration decreases to a very low level and increases again when lysine is added to the diet (5,22). McLaughlan and Illman (23) reported that the plasma lysine level correlated with the dietary lysine level in estimating the amino acid requirements of growing rats. This indicates that the plasma-dietary lysine relationship observed in growing animals must be basically different from that observed in adults. Fisher et al. (16) recently found that the lysine requirement for adults was considerably lower than that reported in earlier studies. Moreover, the lysine requirement for maintenance is considerably lower than that for growth (24).

In general, the plasma concentration of lysine was affected less than those of other EAA by protein deficiency. This may be explained by the following considerations. 1) A normal or rather increased concentration of plasma lysine was demonstrated in starving chick (8,9) and sheep (10) and protein-deprived men (11), in which tissue breakdown was increased. In subjects on gluten diets, tissue proteins may be catabolized to compensate for the poor amino acid composition of the gluten, as indicated by Banks et al. (22). Since, of the EAAs in tissue proteins, lysine is present in the highest concentration (25,26), breakdown of tissue proteins should result in a higher level of lysine than of other EAAs in the free amino acid pool. 2) Free lysine is metabolized in the tissues more slowly than other EAAs (27), and Yamashita and Ashida (28) found that in lysine deficiency, its breakdown decreased to half the control rate.

When protein intake is restricted to less than 30 mgN/kg, plasma EAAs tend to increase gradually with increase in severity of protein depletion and total NEAAs increase further, as shown in Fig. 2. Changes in plasma free amino acids differed above and below 30 mgN/kg of gluten intake. These findings for plasma free amino acids are consistent with our previous findings in nitrogen balance studies (29) that the slopes of regression equations between nitrogen balance and nitrogen intake become steeper below 30 mgN/kg gluten intake. On the basis of data on plasma amino acids and results of nitrogen balance studies, we concluded that a protein intake of 30 mgN/kg was of biological significance. Thus, the relationship between the increased concentration of plasma free amino acids and the efficiency of protein utilization at intakes of less than 30 mgN/kg was studied further. The present study provides further evidence for the nutritional significance of plasma free amino acids in nitrogen balance studies reported previously (29,30).
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