Nitrogen Requirement of Amino Acid Mixture with Maintenance Energy in Young Men

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Summary The effect of energy on nitrogen balance was examined in young men given amino acid mixture. The minimum amino acid nitrogen requirement for nitrogen equilibrium was determined together with the egg protein requirement. In experiment 1, the nitrogen sparing effect of energy was evaluated in four male students receiving diet containing an amino acid mixture and a constant nitrogen intake of 3.5 g N/day, which was equivalent to the nitrogen requirement with excess energy intake determined by Rose and Wixom (1). When the dietary energy supply was 45 kcal/kg, which is approximately the maintenance level, the mean nitrogen balance was negative, being $-23.9\pm9.3$ mg N/kg. However, with an excess energy intake of 55 kcal/kg, the nitrogen balance improved significantly, being $-6.1\pm7.7$ mg N/kg. In experiment 2, the nitrogen requirement of egg-pattern amino acid mixture for apparently zero balance was evaluated at maintenance energy intake in 28 Japanese young men and was compared with that of egg protein. After receiving standard diet, the subjects were given a semi-purified experimental diet containing egg-pattern amino acid mixture at nitrogen intake levels of 60, 75, 100, and 130 mg N/kg for two weeks. Then all groups except the 60 mg N/kg group were given isonitrogenous egg protein diet for another week. Energy intake was kept constant at approximately the maintenance level of $44.4\pm1.4$ kcal/kg throughout the experiment. Nitrogen balance was not significantly different in groups given egg-pattern amino acid mixture and intact egg protein in each nitrogen intake level. From regression analysis, the nitrogen requirement for nitrogen equilibrium of the amino acid mixture was calculated to be $110.1\pm50.2$ mg N/kg, which was not significantly different from the value of $88.4\pm40.6$ mg N/kg of egg protein. It was concluded that the total amino acid requirement estimated by Rose and Wixom (1) was too low because they gave excess energy, and that there was no difference between the nitrogen requirement of egg protein.
and that of the corresponding amino acid mixture.

**Key Words** amino acid requirement, maintenance energy, amino acid mixture, egg protein pattern, nitrogen balance

Studies on amino acid requirements have been carried out mainly on requirements of individual essential amino acids and there have been few studies on the total amino acid nitrogen requirement except for those of Rose and Wixom (1). Rose and Wixom (1) estimated the amino acid nitrogen requirement to be 3.5 g N/day (50 mg N/kg for 70 kg body weight) with an excess energy intake of 55 kcal/kg. But this is considerably lower than the value of 90 mg N/kg of egg protein obtained in our laboratory with an approximate maintenance energy intake of 45 kcal/kg (2). It was necessary to investigate whether the difference between the total amino acid requirement determined by Rose and Wixom (1) and that of egg protein determined by us (2) depended on the difference in energy intake or in the nitrogen source. There have been some studies on nutritional differences between intact proteins and corresponding amino acid mixtures. Rose et al. (3) reported that the nutritive value of amino acid mixture with the pattern of casein was inferior to that of intact casein and that with the mixture excess energy above the maintenance intake was required to obtain a similar nitrogen balance to that with intact protein. But Anderson et al. (4) reported that the nitrogen balance with an amino acid mixture simulating casein was not significantly different from that with intact casein. Thus no definite conclusion has been reached on whether the nutritive value of an amino acid mixture is different from that of intact protein. The protein sparing effect of energy has been known for a long time (5, 6). Inoue et al. (2) and Kishi et al. (7) found that the egg protein nitrogen requirement was reduced by 1.9 mg N/kcal on increasing the energy intake from a maintenance level of 45 kcal/kg to an excess level of 57 kcal/kg. Furthermore, Inoue et al. (8) showed an interrelation of nitrogen intake, energy intake, and nitrogen balance by multiple regression analysis. But there are no reports of quantitative examinations of the nitrogen sparing effect of energy with amino acid mixture diet. In this work, therefore, we first examined the effect of energy intake on nitrogen balance with an amino acid mixture containing 3.5 g N/day as total nitrogen, which is equivalent to the total amino acid nitrogen requirement determined by Rose and Wixom (1). Then in experiment 2 we examined the amino acid nitrogen requirement under maintenance energy conditions with an egg-pattern amino acid mixture and compared results with that for egg protein to determine whether there was any nutritional difference between the two nitrogen sources.

**MATERIALS AND METHODS**

Thirty two male Japanese students of 19 to 30 years old in this university, participated in this study. During the experiments they lived in a metabolic ward
under our clinical supervision and were weighed daily before breakfast after voiding urine. During experiments, they continued normal daily routines, but refrained from any hard physical activities. Table 1-1 and 1-2 summarizes the characteristics of these subjects and their energy and nitrogen intakes.

Subjects were given standard diet for five to seven days before experiments. Standard diet was prepared from conventional foodstuffs containing about 1.25g of protein per kg (200mg N/kg) and about 45kcal/kg of approximate maintenance energy. These nitrogen and energy intakes were enough to meet the allowance levels for Japanese men (9), and the subjects maintained constant weight during the period on standard diet as described later. After the standard period, the subjects were given a semi-purified experimental diet.

Experiment 1. We investigated whether energy had a nitrogen sparing effect with amino acid mixture diet as with protein diet. During the experiment, four subjects were given semi-purified experimental diet containing amino acid mixture as the sole nitrogen source. The energy intake was 45 kcal/kg, or approximately the maintenance energy level, for ten days and 55 kcal/kg, or an excess energy level, for ten days, with a three-day recovery period between these periods. During the recovery period, the subjects ate conventional Japanese diet ad libitum. The 45 kcal/kg diet and 55 kcal/kg diet were given according to a simple switchback schedule; namely, two of four subjects were fed 45 kcal/kg diet for the first ten day period and then 55 kcal/kg diet for another ten days and the other two subjects were given the two diets in the reverse order. For comparison of the requirement with that reported by Rose et al. (1, 10), the same total amino acid nitrogen and essential amino acid nitrogen levels as the requirements reported by them were supplied throughout the two dietary periods; that is, 3.5g N/day of total nitrogen and 1.46g N/day of essential amino acid nitrogen. The compositions of essential and nonessential amino acids in the mixture simulated those in egg protein, as shown in Table 2, but cystine and tyrosine were replaced by isonitrogenous amounts of methionine and phenyl-

Vol. 29, No. 2, 1983

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Table 1-1. Characteristics of subjects and their energy and nitrogen intakes in experiment 1.

| Subject | Age | Weight\(^a\) (kg) | Height (cm) | Energy intake | N intake |
|---------|-----|------------------|-------------|---------------|---------|
|         |     |                  |             | Maintenance\(^b\) (kcal/kg) | Excess\(^b\) (kcal/kg) | St (mg N/kg) | Exp\(^c\) (mg N/kg) |
| 1       | 24  | 69.5             | 170         | 44.7          | 54.6    | 201.4 | 50.4 |
| 2       | 21  | 62.4             | 172         | 44.7          | 54.6    | 198.7 | 56.1 |
| 3       | 23  | 51.6             | 163         | 44.5          | 54.4    | 197.7 | 67.8 |
| 4       | 23  | 71.2             | 168         | —             | 54.8    | 199.4 | 49.2 |

\(^a\) Mean body weight during standard period (St). \(^b\) Maintenance: 45 kcal/kg/day, Excess: 55 kcal/kg/day. \(^c\) The daily total nitrogen intake during the two experimental periods was constant at 3.5 g.
Table 1-2. Characteristics of subjects and their energy and nitrogen intakes in experiment 2.

| No. | Age | Weight (kg) | Height (cm) | Energy intake | N intake |
|-----|-----|-------------|-------------|---------------|----------|
|     |     |             |             | St $^a$ | AA $^a$ (kcal/kg) | Egg$^a$ | St | Exp$^a$ |
|     |     |             |             |         |               |         |     |        |
| 60 mg N/kg |    |             |             | 45.0 | 45.0 | — | 199.8 | 59.5 |
| 101 22 | 72.1 | 171 |             | 45.0 | 45.0 | — | 198.2 | 59.5 |
| 102 23 | 63.6 | 171 |             | 44.6 | 44.6 | — | 196.1 | 58.8 |
| 103 21 | 77.5 | 160 |             | 42.2 | 40.9 | — | 197.0 | 59.1 |
| 104 19 | 59.9 | 168 |             | 44.3 | 44.3 | — | 198.6 | 59.6 |
| 105 23 | 76.5 | 162 |             | 42.7 | 42.7 | — | 199.8 | 74.9 |
| 75 mg N/kg |    |             |             | 45.0 | 45.0 | 45.0 | 199.8 | 74.9 |
| 201 22 | 63.1 | 171 |             | 44.1 | 42.9 | 41.8 | 197.8 | 74.2 |
| 202 20 | 72.8 | 160 |             | 44.6 | 44.6 | 44.6 | 198.0 | 74.3 |
| 203 24 | 57.6 | 174 |             | 45.1 | 45.1 | 45.1 | 200.3 | 75.1 |
| 204 19 | 63.9 | 174 |             | 46.2 | 46.8 | 46.8 | 203.5 | 76.3 |
| 205 21 | 52.1 | 172 |             | 44.6 | 44.6 | 44.6 | 198.1 | 74.3 |
| 206 23 | 63.6 | 171 |             | 41.2 | 40.8 | 40.6 | 197.8 | 74.2 |
| 207 21 | 75.8 | 160 |             | 44.5 | 44.6 | 43.8 | 199.0 | 74.6 |
| 208 22 | 59.3 | 168 |             | 44.6 | 44.6 | 44.6 | 198.1 | 74.3 |
| 209 22 | 56.6 | 165 |             | 43.8 | 44.8 | 45.2 | 203.5 | 75.3 |
| 210 23 | 72.7 | 162 |             | 45.2 | 45.9 | 46.2 | 200.7 | 100.3 |
| 100 mg N/kg |    |             |             | 45.2 | 45.6 | 45.2 | 200.8 | 100.4 |
| 301 22 | 71.8 | 171 |             | 42.0 | 42.5 | 42.5 | 195.3 | 98.9 |
| 302 23 | 49.8 | 168 |             | 42.0 | 42.5 | 42.5 | 195.3 | 99.4 |
| 303 21 | 77.8 | 160 |             | 44.7 | 44.9 | 45.7 | 198.8 | 99.8 |
| 304 21 | 59.4 | 171 |             | 44.2 | 44.9 | 44.9 | 196.5 | 99.8 |
| 305 20 | 64.1 | 168 |             | 44.7 | 45.0 | 45.0 | 200.2 | 100.1 |
| 306 26 | 64.1 | 172 |             | 44.2 | 44.9 | 44.9 | 196.5 | 99.8 |
| 307 30 | 55.9 | 153 |             | 45.0 | 45.0 | 45.0 | 200.2 | 100.1 |
| 308 23 | 63.0 | 172 |             | 45.0 | 45.6 | 45.0 | 199.9 | 100.0 |
| 130 mg N/kg |    |             |             | 45.2 | 45.2 | 45.2 | 200.8 | 130.5 |
| 401 23 | 57.8 | 173 |             | 45.2 | 45.0 | 45.0 | 199.9 | 130.0 |
| 402 23 | 57.0 | 166 |             | 45.0 | 45.0 | 45.0 | 199.7 | 129.8 |
| 403 19 | 66.1 | 173 |             | 44.5 | 43.2 | 42.9 | 200.0 | 130.0 |
| 404 20 | 47.0 | 159 |             | 45.0 | 45.0 | 45.0 | 198.6 | 130.0 |

*$^a$St, standard diet period; AA, amino acid diet period; Egg, egg protein diet period; Exp, amino acid diet period and egg protein diet period.

alanine, respectively. The composition of the experimental diet is given in Table 3. Energy was supplied mainly as cornstarch, sucrose, dextrin, corn oil and shortening. Dietary fat supplied about 30% of the total energy intake. Vitamin and mineral mixtures given in the experimental periods fully satisfied the allowances of Japanese men (2). In addition, 5 g of agar was given as roughage.

The mean urinary nitrogen excretions in the last four days of each experimental

*J. Nutr. Sci. Vitaminol.*
Table 2. Composition of amino acid mixture simulating whole egg protein.a

|                | As used (g/day) | N content (mg/day) |
|----------------|----------------|--------------------|
| **EAA**        |                |                    |
| Ile            | 2.90           | 310                |
| Leu            | 4.06           | 434                |
| Lys·HCl        | 4.01           | 615                |
| Met            | 2.94           | 276                |
| Phe            | 4.38           | 371                |
| Thr            | 2.36           | 278                |
| Trp            | 0.69           | 95                 |
| Val            | 3.15           | 377                |
| **Total**      | 24.49          | 2,756b             |
| **NEAA**       |                |                    |
| Arg            | 2.81           | 904                |
| His            | 1.12           | 303                |
| Ala            | 2.72           | 428                |
| Asp            | 4.43           | 466                |
| Glu            | 5.86           | 558                |
| Gly            | 1.52           | 284                |
| Pro            | 1.92           | 234                |
| Ser            | 3.52           | 469                |
| **Total**      | 23.90          | 3,646              |
| **Grand total**| 48.39          | 6,402              |

a Intake is given for a 64 kg subject (code No. 305) receiving 100 mg N/kg diet. b The content of essential amino acid nitrogen with the egg pattern is 43% of the total nitrogen. In experiment 1, the essential amino acid nitrogen supplied was equivalent to the requirement estimated by Rose et al. (10) and was 41.8% of the nitrogen intake of 3.5 g/day. c In some cases, arginine and histidine were used as chloride forms.

period were used to estimate nitrogen balance. Apparent nitrogen balances were calculated from the nitrogen intake and fecal and urinary excretions. Subject 4 did not participate in the study with 45 kcal/kg diet because he had a cold.

Experiment 2. In this experiment, the apparent nitrogen requirement of egg-pattern amino acid mixture was evaluated under approximate maintenance energy conditions and was compared with that of egg protein. After a period on standard diet, subjects were fed a semi-purified experimental diet containing amino acid mixture simulating whole egg protein as a sole nitrogen source for two weeks. The nitrogen intakes during the period on amino acid mixture were 60 mg N/kg for five subjects, 75 mg N/kg for ten, 100 mg N/kg for eight, and 130 mg N/kg for five subjects. For comparison of the nutritional value of egg-pattern amino acid mixture with that of egg protein, all groups except that receiving 60 mg N/kg were given egg protein diet for one week after amino acid mixture. Energy intake throughout the experimental period was kept constant at approximately the maintenance intake of

Vol. 29, No. 2, 1983
Table 3. Composition of experimental diet.

|                        | Experiment 1<sup>a</sup> |  |  |      | Experiment 2<sup>b</sup> |  |  |  |
|------------------------|--------------------------|---|---|-----|--------------------------|---|---|---|
|                        | Maintenance             | Excess       | Amino acid   | Egg protein              |                  |  |  |
|                        | energy diet              | energy diet  | diet          | diet                      |                  |  |  |
|                        | (g)                      | (g)          | (kcal)        | (kcal)                    |                  |  |  |
| Amino acid mix.        | 26.9                     | 88           | 26.9          | 88                        | 48.4            | 160|   |
| Whole egg              | —                        | —            | —             | —                         | —                | — | — |
| Cornstarch             | 156                      | 552          | 180           | 637                       | 129             | 457| 105|
| Sucrose                | 94                       | 364          | 137           | 530                       | 84              | 325| 84 |
| Dextrin<sup>d</sup>    | 282                      | 1,072        | 379           | 1,440                     | 357             | 1,357|319|1,212|
| Corn oil               | 20                       | 177          | 20            | 177                       | 40              | 354 | 30 | 265|
| Shortening             | 60                       | 530          | 60            | 530                       | 25              | 221 | 20 | 177|
| Agar                   | 5                        | —            | 5             | —                         | 5               | — | — |
| Baking powder          | 3                        | 4            | 3             | 4                         | 4               | 5 | 4 | 5 |
| NaCl                   | 5                        | —            | 5             | —                         | 5               | — | — | — |
| Vitamin mix.<sup>e</sup> | 3                     | —            | 3             | —                         | 3               | — | 3 | — |
| Mineral mix.<sup>e</sup> | 6                     | —            | 6             | —                         | 6               | — | 6 | — |
| Total                  | 2,791                    | 3,410        | 2,879         | 2,881                     |                  |  |  |  |

<sup>a</sup> Intakes are for a 62 kg subject (code No. 2). <sup>b</sup> Intakes are for a 64 kg subject (code No. 305) receiving 100 mg N/kg and 45 kcal/kg diet. <sup>c</sup> Cornstarch, sucrose, and dextrin were added to maintenance energy diet. <sup>d</sup> "Kona-ame," Hayashibara & Co., Ltd., Okayama, Japan. <sup>e</sup> For details, see Reference (2).

about 45 kcal/kg. The amino acid pattern was based on that of egg protein (11) but cystine and tyrosine were replaced by isonitrogenous amounts of methionine and phenylalanine, respectively (Table 2). The composition of the experimental diet is shown in Table 3.

Urine was collected every 24 hr and feces in each dietary period were pooled. Apparent nitrogen balances were calculated from intakes and fecal and average urinary nitrogen excretions in the last five and three days of periods on amino acid and egg protein diets, respectively. The total nitrogen contents of urine and feces were analyzed by the semimicro-Kjeldahl method (12). Urinary creatinine was determined by the Folin method as modified by Koishi (13). Urinary and plasma urea was analyzed by the urease-indophenol method (14). Urinary α-amino nitrogen was measured by the method of Lorentz and Flatter (15). Venous blood samples were taken before breakfast at the end of each dietary period for routine hematologic examinations and measurements of urea nitrogen, total protein, and albumin. Free amino acids in the plasma were determined with an automatic amino acid analyzer (Model A-3300, Irica Mgf. Co., Ltd., Kyoto, Japan).
Table 4. Effects of energy intake on body weight, nitrogen excretion, and nitrogen balance.

|                | Initial wt. (kg) | Final wt. | N intake | Fecal N (mg N/kg) | Urinary N (mg N/kg) | N balance |
|----------------|-----------------|-----------|----------|-------------------|---------------------|-----------|
| Maintenance\(^a\) | 61.2            | 60.2      | 58.1     | 12.3              | 69.7                | -23.9     |
| (3)\(^b\)      | 8.6             | 9.4       | 8.9      | 0.3               | 6.9                 | 9.3       |
| Excess\(^c\)   | 63.6            | 63.4      | 55.9     | 12.8              | 49.2                | -6.1\(^d\) |
| (4)            | 8.9             | 9.1       | 8.5      | 1.1               | 13.2                | 7.7       |

\(^a\) Maintenance energy intake of 45 kcal/kg body weight. \(^b\) Numbers in parentheses are numbers of subjects. \(^c\) Excess energy intake of 55 kcal/kg body weight. \(^d\) Significant difference from values in maintenance energy period. \(^*\) p < 0.05.

RESULTS

Experiment 1

The body weight did not change during the first week when subjects received the standard diet with adequate protein and an approximate maintenance energy level of 45 kcal/kg. Changes in body weight, nitrogen intake and excretion, and nitrogen balance in the two experimental periods are given in Table 4. During the experimental period, the body weight tended to decrease gradually when a maintenance energy level of 45 kcal/kg was given, but was maintained with an excess energy level of 55 kcal/kg, except in one subject (No. 2).

Urinary nitrogen excretion during the excess energy period was 49.2 ± 13.2 mg N/kg, while that during the maintenance energy period was 69.7 ± 6.9 mg N/kg. Fecal nitrogen excretion was similar in the two energy periods, that is, 12.3 ± 0.3 mg N/kg during the maintenance energy period and 12.8 ± 1.1 mg N/kg during the excess energy period. Nitrogen balance was negative (−23.9 ± 9.3 mg N/kg) with maintenance energy, but improved significantly (−6.1 ± 7.7 mg N/kg) with excess energy.

Experiment 2

When the subjects were given a standard diet with an approximate maintenance energy intake of 44.4 ± 1.1 kcal/kg and nitrogen intake of 199.0 ± 2.0 mg N/kg, their body weight did not change significantly, as shown in Fig. 1. On changing to low nitrogen diet with amino acid mixture, the body weights of all groups decreased. The losses of body weight during the period on amino acid mixture were marked, especially in groups with higher nitrogen intakes of 100 and 130 mg N/kg. On changing to egg protein diet, the body weight increased temporarily and then decreased again.

The mean values for urinary total nitrogen in the last five and three days of the
Fig. 1. Effect of nitrogen sources on daily changes in body weight at various levels of nitrogen intake. Nitrogen intake: 60 (△), 75 (□), 100 (○), and 130 (●) mg N/kg. Values are difference from the mean body weight during the standard period.

Table 5. Nitrogen intake, nitrogen excretion and nitrogen balance during amino acid and egg protein periods.

| Amino acid diet | Egg protein diet |
|-----------------|-----------------|
|                | NI              | FN               | UN               | NB   | NI              | FN             | UN               | NB   |
|                | (mg/kg)         | (mg/kg)          | (mg/kg)          |      | (mg/kg)         | (mg/kg)        | (mg/kg)          |      |
| 60 mg N/kg (5)  | Mean            | 59.4             | 10.2             | 57.7 | -8.5            | 74.8           | 12.2             | 64.9 | -2.3            |
|                 | SD              | 0.4              | 1.1              | 5.6  | 6.0             | 0.7            | 1.3              | 7.9  | 11.4            |
| 75 mg N/kg (10) | Mean            | 74.8             | 10.4**           | 69.2 | -4.8            | 74.8           | 15.4             | 64.9 | -2.3            |
|                 | SD              | 0.7              | 1.1              | 7.9  | 7.6             | 0.7            | 1.3              | 11.4 | 10.8            |
| 100 mg N/kg (8) | Mean            | 99.8             | 11.8**           | 87.7 | +0.3            | 99.8           | 15.4             | 84.2 | +0.3            |
|                 | SD              | 0.5              | 1.8              | 5.6  | 5.5             | 0.5            | 2.5              | 7.2  | 7.8             |
| 130 mg N/kg (5) | Mean            | 130.1            | 10.0**           | 118.9| +1.2            | 130.1          | 13.6             | 105.5| +11.0           |
|                 | SD              | 0.3              | 1.4              | 10.0 | 11.1            | 0.3            | 2.5              | 4.9  | 7.0             |

aNI, nitrogen intake; FN, fecal nitrogen; UN, urinary nitrogen; NB, nitrogen balance.  
bNumbers in parentheses are numbers of subjects.  
cSignificant difference from value in egg protein period. **p<0.01.

period on amino acid mixture and egg protein, respectively, are given in Table 5. Urinary total nitrogen excretion decreased rapidly to fairly steady levels within a week on changing to the amino acid diet from standard diet, as shown in Fig. 2. There was no significant difference between the mean urinary nitrogen excretions in the two periods, as shown in Table 5.

Although urinary urea excretion was similar in the two dietary periods,
ammonia excretion was significantly higher in the period on amino acid diet than in that on egg protein diet, as shown in Table 6. In the group given 130 mg N/kg, though ammonia nitrogen excretion was 9.3% of the total nitrogen excretion during the period on egg protein, it increased markedly to 15.8% during the period on amino acid diet. But the percentages of total nitrogen excretion as urea plus ammonia were similar with the two nitrogen sources. The increase of ammonia excretion seemed to be the result of giving lysine, histidine, and arginine in their chloride forms (16). Urinary \(\alpha\)-amino nitrogen excretion was not affected by the nitrogen intake or nitrogen source. Urinary creatinine during the period with low nitrogen intake was significantly lower than that during the period on standard diet.

The fecal nitrogen excretions in each period are given in Table 5. During the period on amino acid diet, the mean fecal nitrogen excretion for all subjects was \(10.7 \pm 1.5\) mg N/kg and the value was not affected by the nitrogen intake. The value of \(10.7\) mg N/kg is slightly lower than that of \(12.7\) mg N/kg of metabolic fecal nitrogen obtained for Japanese adult men by Inoue et al. (17). Therefore, amino acid nitrogen was concluded to be absorbed completely. On the other hand, the mean value for fecal nitrogen loss in all subjects during the period on egg protein diet was \(13.6 \pm 2.4\) mg N/kg, which was significantly higher than that during the period on amino acid diet. The true digestibilities of egg protein nitrogen were \(99.7 \pm 0.6\), \(97.2 \pm 2.4\), and \(98.8 \pm 0.8\) at intakes of 75, 100, and 130 mg N/kg, respectively.

Values for nitrogen balance are given in Table 5 and Fig. 3. Except in the 60 mg N/kg group, nitrogen balances at all nitrogen intakes were not significantly


Table 6. Urinary constituents during standard and experimental periods (Experiment 2).

|       | St. diet         | A.A. diet        | Egg protein diet |
|-------|------------------|------------------|-----------------|
|       |                  |                  |                 |
| 60 mg N/kg |                  |                  |                 |
| (5)   |                  |                  |                 |
| Total N | 151.8 ± 11.7b    | 57.7 ± 5.6       |                 |
| Urea N | 123.7 ± 11.4     | 34.6 ± 4.8       |                 |
| Ammonia N | 8.0 ± 0.6       | 7.8 ± 1.0        |                 |
| Amino N | 7.2 ± 0.1        | 5.4 ± 0.3        |                 |
| Creatinine N | 10.3 ± 0.6    | 9.0 ± 0.6 oo     |                 |
| 75 mg N/kg |                  |                  |                 |
| (10)  |                  |                  |                 |
| Total N | 164.1 ± 12.7    | 69.2 ± 7.9       | 64.9 ± 11.4     |
| Urea N | 135.8 ± 12.7     | 43.6 ± 8.0       | 44.7 ± 10.9     |
| Ammonia N | 8.6 ± 0.8       | 10.1 ± 1.2***d  | 5.9 ± 0.6       |
| Amino N | 6.6 ± 0.7        | 5.1 ± 1.1*       | 5.8 ± 1.9       |
| Creatinine N | 10.5 ± 0.7    | 9.5 ± 0.6 oo     | 9.6 ± 0.8 oo    |
| 100 mg N/kg |                  |                  |                 |
| (8)   |                  |                  |                 |
| Total N | 157.7 ± 6.5      | 87.7 ± 5.6       | 84.2 ± 7.2      |
| Urea N | 129.4 ± 6.8      | 61.2 ± 6.1       | 61.8 ± 9.9      |
| Ammonia N | 8.3 ± 1.0        | 10.0 ± 1.9**     | 7.1 ± 1.5       |
| Amino N | 6.5 ± 0.6        | 4.9 ± 0.4*       | 6.2 ± 1.4       |
| Creatinine N | 10.3 ± 1.0    | 9.5 ± 1.1 oo     | 9.5 ± 0.7 oo    |
| 130 mg N/kg |                  |                  |                 |
| (5)   |                  |                  |                 |
| Total N | 161.7 ± 18.0     | 118.9 ± 10.0     | 105.5 ± 4.9     |
| Urea N | 133.1 ± 17.8     | 83.6 ± 8.6       | 79.4 ± 5.1      |
| Ammonia N | 8.6 ± 0.9        | 18.8 ± 1.1***    | 9.8 ± 0.9       |
| Amino N | 6.7 ± 0.3        | 5.5 ± 0.5***     | 4.7 ± 0.4       |
| Creatinine N | 10.4 ± 1.2    | 10.1 ± 1.0***oo | 9.8 ± 1.1 oo    |

Numbers in parentheses are numbers of subjects. Figures are mean ± SD (mg N/kg) in the last 3, 5, and 3 days of standard, amino acid mixture, and egg protein periods, respectively. Significant difference from value with standard diet. *p<0.05, **p<0.01. Significant difference from value with egg protein diet. *p<0.05, **p<0.01.

Different in the two dietary periods. Nitrogen balance improved with increase in nitrogen intake. Significant rectilinear relations were found between nitrogen intake (X, mg N/kg) and apparent nitrogen balance (Y, mg N/kg) for both amino acid mixture and egg protein as shown in the following equations:

Amino acid mixture: \[ Y = 0.140X - 15.38 \]  
\( (n=28, r=0.422, p<0.05) \)

Egg protein: \[ Y = 0.225X - 20.02 \]  
\( (n=23, r=0.481, p<0.05) \)

However, no significant difference was found between the two regression lines by the statistical analyses using F-test on variance and t-tests on slope and y-intercept. From the above equations, the maintenance intake of amino acid mixture with egg

*J. Nutr. Sci. Vitaminol.*
Fig. 3. Relation between nitrogen intake and nitrogen balance during the two dietary periods with egg-pattern amino acid mixture and egg protein with maintenance energy intake. ○, egg-pattern amino acid mixture; ●, egg protein.

Table 7. Net protein utilizations of amino acid mixture and whole egg protein at each nitrogen level.

| Nitrogen Level (mg N/kg) | Amino acid mix. | Egg protein |
|-------------------------|-----------------|-------------|
| 60                      | 63.1 ± 10.5b    | 58.6 ± 14.8 |
| 75                      | 55.2 ± 10.3     |             |
| 100                     | 46.4 ± 5.5      | 46.3 ± 7.8  |
| 130                     | 36.3 ± 8.5      | 43.9 ± 5.5  |

*Number of subjects in parentheses. b Mean ± SD.

pattern for apparent nitrogen equilibrium was calculated to be 110.1 ± 52.8 mg N/kg, and that of egg protein to be 88.8 ± 40.6 mg N/kg. There was no significant difference between the two values. Net protein utilization (NPU) tended to decrease with increase in nitrogen intake, as shown in Table 7. The NPU for amino acid mixture was not significantly different from that for egg protein.

The data on whole blood and plasma are shown in Table 8. No consistent alterations were observed in any constituents except plasma urea nitrogen. The mean plasma urea nitrogen of all subjects during the experimental period with low nitrogen intake was 6.9 ± 1.9 mg N/100 ml, while that during the standard period with adequate nitrogen intake was 12.3 ± 2.6 mg N/100 ml. The concentrations of free amino acids in the plasma are given in Table 9. Total essential amino acids in the plasma decreased significantly during the experimental period except in the group given 130 mg N/kg, but there were no significant differences between the levels with the two nitrogen sources. The concentration of total nonessential amino acids...
Table 8. Data on blood analysis in experiment 2.

|                | St. diet (28)a | Amino acid diet | Egg protein diet |
|----------------|---------------|-----------------|-----------------|
|                | 60 (5)        | 75 (10)         | 100 (8)         | 130 (5) |
| Whole blood    |               |                 |                 |
| S.G. b         | Mean 1.056    | 1.055           | 1.055           | 1.057 |
|                | SD 0.002      | 0.001           | 0.001           | 0.002 |
| Ht. b          | Mean 43.9     | 44.0            | 42.7            | 44.6  |
|                | SD 2.0        | 1.3             | 1.9             | 1.7   |
| RBC b (× 10^6) | Mean 458      | 465             | 459             | 463   |
|                | SD 37         | 21              | 34              | 24    |
| WBC b          | Mean 5,470    | 4,620           | 5,430           | 5,830 |
|                | SD 950        | 750             | 1,360           | 980   |
| Hb. b          | Mean 14.9     | 14.2            | 14.5            | 15.4  |
|                | SD 0.9        | 0.7             | 1.1             | 0.6   |
| Plasma         |               |                 |                 |
| S.G. b         | Mean 1.025    | 1.026           | 1.026           | 1.025 |
|                | SD 0.001      | 0.003           | 0.001           | 0.001 |
| T.P. b         | Mean 6.96     | 6.68            | 6.91            | 6.69  |
|                | SD 0.58       | 0.36            | 0.47            | 0.20  |
| Alb. b         | Mean 4.36     | 4.19            | 4.41            | 4.11c |
|                | SD 0.26       | 0.05            | 0.16            | 0.14  |
| Urea N b       | Mean 12.3     | 5.9c            | 6.2**          | 8.1c  |
|                | SD 2.6        | 2.2             | 2.2             | 1.7   |

a Number of subjects. b S.G., specific gravity; Ht., hematocrit; RBC, red blood cell; WBC, white blood cell; Hb., hemoglobin (g/100 ml); T.P., total protein (g/100 ml); Alb., albumin (g/100 ml); Urea N, (mg N/100 ml). c Significant difference from value in standard diet period. ** p<0.01.

eg acids was highest in the 60 mg N/kg group, being 2,211 ± 75 μmol/liter. The E/N ratio decreased with decrease in nitrogen intake during both dietary periods.

DISCUSSION

Rose and Wixom (1) estimated the nitrogen requirement of an amino acid mixture for apparent nitrogen equilibrium as 3.5 g N/day (50 mg N/kg as 70 kg body weight) with a large excess energy intake of 55 kcal/kg body weight. This value for amino acid nitrogen requirement is lower than the egg protein requirement with a maintenance energy intake estimated by us and others. For instance, Calloway and
|                | St. diet | Amino acid diet | Egg protein diet |
|----------------|----------|-----------------|-----------------|
|                | 200 (25) | 60 (5)          | 75 (5)          | 100 (7) | 130 (5) | 75 (5) | 100 (7) | 130 (3) |
| **EAA**        |          |                 |                 |         |         |        |         |        |
| Thr            | 138 ± 26b | 142 ± 28       | 139 ± 12**     | 153 ± 18*** | 187 ± 12*** | 116 ± 16 | 121 ± 17 | 156 ± 46 |
| Val            | 250 ± 43  | 221 ± 21       | 199 ± 28***    | 216 ± 38 | 229 ± 29 | 214 ± 24 | 220 ± 46 | 211 ± 52 |
| Cys            | 76 ± 27   | 54 ± 9         | 55 ± 8         | 55 ± 11  | 44 ± 4*   | 61 ± 9   | 58 ± 8   | 70 ± 10  |
| Met            | 41 ± 14   | 38 ± 10        | 41 ± 13        | 41 ± 12  | 51 ± 6*   | 42 ± 6   | 43 ± 15  | 38 ± 8   |
| Ile            | 78 ± 17   | 64 ± 5         | 69 ± 15        | 64 ± 7   | 78 ± 15   | 56 ± 7   | 70 ± 12  | 70 ± 6   |
| Leu            | 134 ± 20  | 112 ± 10°      | 104 ± 11°      | 103 ± 9** | 117 ± 11  | 101 ± 8** | 113 ± 13° | 131 ± 15 |
| Tyr            | 64 ± 17   | 56 ± 3         | 58 ± 9         | 49 ± 5   | 72 ± 10   | 54 ± 5   | 61 ± 14  | 62 ± 8   |
| Phe            | 71 ± 18   | 65 ± 5         | 56 ± 2         | 65 ± 7   | 84 ± 10   | 57 ± 3   | 62 ± 7   | 77 ± 20  |
| Lys            | 182 ± 47  | 187 ± 11       | 181 ± 34       | 196 ± 24 | 210 ± 20  | 190 ± 33 | 199 ± 55 | 241 ± 43 |
| **Total**      | 1,035 ± 95 | 939 ± 53°      | 902 ± 38°      | 942 ± 69° | 1,072 ± 58 | 892 ± 55° | 946 ± 89° | 1,056 ± 145 |
| **NEAA**       |          |                 |                 |         |         |        |         |        |
| Ser            | 133 ± 31  | 115 ± 21       | 118 ± 16       | 112 ± 17 | 167 ± 21° | 112 ± 12 | 111 ± 20 | 158 ± 29 |
| Gly            | 241 ± 51  | 319 ± 77°      | 247 ± 48       | 280 ± 31* | 349 ± 51° | 262 ± 36 | 260 ± 42 | 287 ± 19 |
| Ala            | 425 ± 136 | 646 ± 72°      | 485 ± 72       | 569 ± 53° | 599 ± 103° | 547 ± 85 | 553 ± 75° | 524 ± 42 |
| His            | 99 ± 21   | 54 ± 11        | 91 ± 5         | 89 ± 11  | 135 ± 15* | 91 ± 6   | 91 ± 13  | 107 ± 8  |
| Arg            | 103 ± 26  | 56 ± 11        | 83 ± 15*       | 82 ± 14* | 122 ± 35  | 72 ± 15| 93 ± 15  | 117 ± 43 |
| Glu + Gln      | 799 ± 97  | 940 ± 50°      | 796 ± 118      | 841 ± 58 | 740 ± 86  | 831 ± 118 | 821 ± 55 | 668 ± 20 |
| **Total**      | 1,800 ± 278 | 2,211 ± 75°   | 1,841 ± 190    | 1,974 ± 69 | 2,111 ± 183° | 1,910 ± 123 | 1,920 ± 131 | 1,858 ± 73 |
| E/N ratio      | 0.58 ± 0.06 | 0.42 ± 0.02°   | 0.49 ± 0.06°   | 0.48 ± 0.03° | 0.51 ± 0.03° | 0.47 ± 0.04° | 0.50 ± 0.07° | 0.57 ± 0.07 |

a Number of subjects. b Values are means ± SD (μmol/liter). c Significant difference from value with egg protein diet; *p<0.05, **p<0.01. d Significant difference from value with standard diet; °p<0.05, °°p<0.01.
Margen (18) reported that the true minimum egg protein requirement was 4.8 g N/day (68 mg N/kg as 70.1 kg body weight), which is more than the endogenous nitrogen output of 3.5 g N/day determined in that study. Young et al. (19) also evaluated the nitrogen requirement of whole egg protein as 60–94 mg N/kg body weight. In addition, Inoue et al. (2) reported that apparent nitrogen equilibrium was established at about 90 mg N/kg of egg protein nitrogen with maintenance energy intake. All these values are considerably higher than the amino acid nitrogen requirement reported by Rose and Wixom (1). We thought that this difference might be due to the difference in energy intakes, not to the difference in nitrogen sources.

The effect of energy intake on protein requirement was studied quantitatively by Inoue et al. (2) and Kishi et al. (7), who found that the nitrogen requirement of egg protein decreased with increase in energy intake; that is, it was 124 mg N/kg at 40.2 kcal/kg, 90 mg N/kg at 45.0 kcal/kg, 82 mg N/kg at 48.2 kcal/kg, and 67 mg N/kg at 57.0 kcal/kg of energy intake. Thus the amount of egg protein nitrogen spared by energy was calculated as 1.9 mg N/kcal on changing the energy intake from an approximate maintenance level of 45 kcal/kg to an excess energy level of 57 kcal/kg. For comparison of results with intact egg protein, we investigated the effect of energy intake on nitrogen balance of an amino acid mixture in experiment 1. Results showed that at 3.5 g N/day of amino acid mixture the nitrogen balance was almost zero with the excess energy intake of 55 kcal/kg used by Rose and Wixom (1), but was clearly negative with approximately the maintenance energy intake of 45 kcal/kg. Results also showed that the nitrogen balance improved by 1.7 mg N/kg with increase from maintenance to excess energy intake. This indicates that the nitrogen sparing effect of energy is similar with the amino acid mixture to that with protein. Thus it appears that the amino acid requirement estimated by Rose et al. is too low because of the nitrogen sparing effect of excess energy.

There has been much controversy over differences in nutritional value of intact proteins and mixtures of their constituent amino acids. Some investigators consider that amino acid mixtures have lower nutritional value. For instance, Rose et al. (3) reported that nitrogen utilization of an amino acid mixture with the pattern of casein was inferior to that of intact casein and subjects failed to achieve nitrogen equilibrium below 45 kcal/kg intake even when they received 10 g/day of nitrogen from a mixture of the amino acids and urea. Thus these workers always carried out investigations on essential amino acid requirements with an excess energy intake of 55 kcal/kg and evaluated the total amino acid nitrogen requirement as 3.5 g N/day. This means that Rose et al. conducted experiments on amino acid requirements assuming that the nutritional value of an amino acid mixture was different from that of protein. Swendseid et al. (20) also suggested a possible difference in the nutritional value of intact protein and amino acid mixture. Recently, Matthews and Payne (21) and Silk et al. (22) indicated that differences in the absorption of free amino acids and peptides might affect the nutritional quality at the whole body level. But Anderson et al. (4) reported that nitrogen balance was similar with amino

J. Nutr. Sci. Vitaminol.
acid mixture to that with intact protein. In experiment 2 of the present study, most subjects had similar nitrogen balances regardless of the nitrogen source, but 3 of 28 subjects had a lower nitrogen balance of over 15 mg N/kg on amino acid diet than on egg protein diet. Our estimated value of 110.1 ± 50.2 mg N/kg for the nitrogen requirement of the amino acid mixture did not differ significantly from that of 88.4 ± 40.6 mg N/kg of egg protein. Thus the nutritional quality of amino acid mixture was not inferior to that of protein in the present conditions. Moreover, we do not think that in studies on amino acid and protein requirements it is necessary to supply excess energy as Rose and Wixom (1) did, because the nitrogen sparing effect of energy was similar with amino acid mixture to that with protein and nitrogen equilibrium could be obtained by increasing the intake of amino acid mixture with a maintenance energy level in the present study.

Of the 110.1 mg N/kg of amino acid mixture required for nitrogen equilibrium in the present study the amount of essential amino acids was 47.3 mg N/kg, as the content of essential amino acid nitrogen of egg-pattern amino acid mixture is 43%. This is apparently higher than the value for the sum of individual amino acid requirements of 20.9 mg N/kg recalculated as 70 kg body weight (1.46 g N/day) and 14.7 mg N/kg obtained by Rose et al. (10) and Inoue et al. (23), respectively. It has been pointed out that more protein is required than is necessary to meet essential amino acid requirements (24). Concerning this problem, Scrimshaw et al. (25) suggested that essential amino acid nitrogen of whole egg protein can be diluted to 21–25% with nonessential amino acid mixture without impairment of the nutritional quality of the protein. Thus, further study is needed to determine the possible difference between the minimum requirement of total essential amino acids determined by the dilution method and the sum of the requirements of individual amino acids.

From the present results, it is supposed that nitrogen utilization of an amino acid mixture was similar to that of the corresponding protein under the present experimental conditions, and that energy had the same nitrogen sparing effect with the amino acid mixture to that observed previously with protein. It was also concluded that Rose and Wixom (1) underestimated the total amino acid nitrogen requirement because they gave excess energy.

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*J. Nutr. Sci. Vitaminol.*
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