Utilization and Requirement of Egg Protein in Japanese Women

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Summary Utilization and requirement of egg protein in Japanese women with two levels of energy intake were estimated. In experiment 1, fifteen female students were given semi-purified diet containing whole egg as the sole nitrogen source for 12 days. Nitrogen intakes were 50 for five, 75 for two and 100 mg N/kg for eight subjects. Habitual energy intake was determined individually by detailed inquiry about the foods consumed before the experiment was started. Mean energy intake was 33 kcal/kg. In experiment 2, eighteen other subjects were given the same diet containing four intake levels of egg protein (30, 50, 75, 100 mg N/kg) with an energy intake of about 100 kcal/day added to that calculated by the food intake inquiry. The mean energy intake was 37 kcal/kg. The total nitrogen contents of the experimental diet, urine and feces were analyzed and the nitrogen balance was estimated from these figures. Significant rectilinear relations were found between nitrogen intake (X , mg N/kg) and balance (Y , mg N/kg). The regression equations were;

Experiment 1: $Y = 0.256X - 34.4$ ($n = 15$, $r = 0.742$)

Experiment 2: $Y = 0.326X - 29.7$ ($n = 18$, $r = 0.645$)

The maintenance intakes of whole egg protein for apparent nitrogen equilibrium were calculated to be 134 and 91 mg N/kg with energy intakes of 33 and 37 kcal/kg, respectively. Net protein utilization (NPU) and digestibility of egg protein were calculated using the obligatory N losses previously determined in Japanese women. The NPUs at the maintenance level of egg protein with energy intakes of 33 and 37 kcal/kg were estimated as 31 and 47, respectively. The mean digestibility of egg protein was 96%.

Key Words protein utilization, protein requirement, egg protein, women, energy intake

The present protein allowance suggested by the FAO/WHO(1) and the Committee on Nutrition (Japan)(2) is based on the amount of dietary protein needed to maintain the nitrogen (N) balance in healthy male adults in laboratory

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tests. The dependence of the N balance on intakes of protein and energy is well known (3-6). Inoue *et al.* (3) and Kishi *et al.* (4) studied the effect of energy intake on protein requirement quantitatively. According to them, the nitrogen requirement of egg protein with maintenance energy intake was 90 mg N/kg and the net protein utilization (NPU) of egg protein at nitrogen equilibrium was 55. Based on these data and obligatory N losses measured in Japanese male subjects, the protein requirement for Japanese adults was estimated (2). However, few reports have been made on obligatory N losses or protein requirement in female subjects. Thus the Committee have been compelled to take the same protein requirement figure for both sexes.

A previous study (7) in our laboratory on obligatory urinary and fecal N losses of Japanese female subjects showed that urinary loss was similar but fecal loss was lower compared with those of male subjects. In this work we estimated utilization and requirement of egg protein in Japanese female subjects at two levels of energy intake.

EXPERIMENTAL

Thirty-three female Japanese students aged 18 to 22 years participated in this study. During the experiments they lived in a metabolic unit and continued their usual activities. Table 1 summarized the characteristics of these subjects.

Subjects were given standard diet for 5 days before the experiments began. Standard diet was prepared from conventional foodstuffs containing about 60 g of protein per day and from the knowledge of approximate energy which was determined individually by detailed inquiry into foods consumed before the experiment. The mean of the energy intake was less than the allowance level for Japanese women, but the subjects maintained a constant weight during the period on standard diet. Thus, the energy intake level was regarded as sufficient to meet its expenditure. After the standard period, the subjects were given a semi-purified experimental diet.

Experiment 1

Fifteen subjects were given semi-purified experimental diet containing whole egg as sole nitrogen source for 12 days. The nitrogen intakes were 50 mg N/kg for five subjects, 75 mg N/kg for two and 100 mg/kg for eight. The energy intake was the same as that in the preceding standard period. The composition of the experimental diet is given in Table 2. Energy was supplied mainly from cornstarch, sucrose and shortening. Dietary fat supplied about 25% of the total energy intake.

The mean urinary nitrogen excretions in the last 4 days of the experimental period were used to estimate nitrogen balance. Apparent nitrogen balances were calculated from the nitrogen intake and fecal and urinary excretions.

Table 1. Characteristics of subjects.

No.	Age (yr)	Height (cm)	Weight ^a (kg)	LBM ^b (kg)	BMR (kcal/kg)
Experiment 1					
101	19	166	63.3	46.0	19.4
102	19	160	55.4	44.2	22.0
103	20	160	58.1	40.4	23.7
104	20	157	57.4	40.3	23.6
105	19	164	50.9	42.1	21.9
106	18	151	56.7	42.4	22.0
107	19	158	55.6	39.8	23.0
108	22	156	54.1	40.3	21.1
109	22	157	51.2	42.7	22.5
110	22	158	57.4	42.4	18.8
111	21	156	46.1	35.5	26.2
112	20	152	49.2	35.0	24.0
113	19	164	52.2	40.6	25.7
114	21	150	43.4	33.9	23.4
115	21	156	51.4	41.0	20.6
Mean	20.1	157.7	53.5	40.4	22.5
SD	1.3	4.7	5.1	3.4	2.1
Experiment 2					
201	19	160	58.4	40.6	21.0
202	21	152	52.2	35.0	22.6
203	21	160	59.0	39.4	17.2
204	20	149	58.8	46.8	18.7
205	21	160	50.1	40.5	24.9
206	21	156	46.3	34.8	20.8
207	21	159	51.9	33.7	20.7
208	21	162	56.1	39.2	19.7
209	21	157	48.2	39.9	19.8
210	20	158	49.2	37.4	21.6
211	20	148	52.3	38.4	22.9
212	19	160	53.9	36.1	19.8
213	19	155	55.1	38.9	20.7
214	18	148	49.3	34.9	20.9
215	19	158	51.2	35.7	22.6
216	19	160	57.0	40.6	21.5
217	20	156	47.2	35.8	19.3
218	18	160	61.2	42.7	22.7
Mean	20.0	156.6	53.1	38.4	21.0
SD	1.1	4.5	4.5	3.3	1.8

^a Mean of 5 days given the standard diet. ^b Calculated from hydrometrically measured body density.

Table 2. Composition of semi-purified experimental diet.^a

Ingredient	Intake (g/day)
Whole egg	250
Cornstarch	180
Sugar	140
Shortening	31
Agar	5
Sodium chloride	3
Mineral mixture ^b	4
Vitamin mixture ^b	2 tablets

^a Intake is given for subject receiving 1,800 kcal and 100 mg N/kg diet. Besides this diet, the subjects consumed black tea, green tea and lemon juice. ^b For details, see Reference (7).

Experiment 2

Eighteen subjects were given the same, semi-purified experimental diet containing four intake levels of whole egg (30, 50, 75, 100 mg N/kg) with an energy intake of about 100 kcal/day added to that of the standard period. Constituents of the diet were the same as those of experiment 1.

Throughout the experiment, subjects were weighed daily before breakfast after voiding urine. Urine was collected every 24 h and feces in the experimental period were pooled. The total nitrogen contents of diet, urine and feces were analyzed by the semimicro-Kjeldahl method. Urinary creatinine was determined by the method of Folin (8). Basal metabolism was measured on a selected day during the experimental period. Lean body mass was calculated from the body density measured hydrometrically by using the equation of Brožek (9).

RESULTS

Energy intake, changes in body weight, urinary creatinine excretion, nitrogen intake and excretion, and nitrogen balance in each subject are given in Table 3. Mean energy intake in experiments 1 and 2 were 33.1 ± 3.5 and 37.3 ± 3.5 kcal/kg, respectively. When the subjects were given the standard diet with approximate maintenance energy intake determined by inquiry into food consumption and with a protein intake of 60 g/day, their body weights did not change significantly. Mean body weight throughout the 5-day period was regarded as the initial weight of each subject. On changing to semi-purified experimental diet, the body weights of all subjects in experiment 1 decreased, while those of the subjects in experiment 2 were maintained or decreased slightly. Urinary creatinine excretion was constant throughout the experimental period in all subjects.

In both experiments, urinary nitrogen excretion decreased rapidly to fairly

Fig. 1. Relation between nitrogen intake and nitrogen balance in female subjects given egg protein with energy intakes of 33 kcal/kg (●) and 37 kcal/kg (○).

steady levels within a week of changing to the experimental diet from the standard diet. Nitrogen balance improved with an increase in nitrogen intake in both experiments 1 and 2. Significant rectilinear relations were found between nitrogen intake (X , mg N/kg) and balance (Y , mg N/kg) as shown in Fig. 1. The regression equation are as follows:

Experiment 1: $Y = 0.256X - 34.4$ ($n = 15$, $r = 0.742$)

Experiment 2: $Y = 0.326X - 29.7$ ($n = 18$, $r = 0.645$)

From the above equations, the maintenance intakes of whole egg protein for apparent nitrogen equilibrium were calculated to be 134 and 91 mg N/kg with energy intakes of 33 and 37 kcal/kg, respectively.

NPU and digestibility of egg protein were calculated using the obligatory urinary and fecal N losses (32.3 and 10.1 mg N/kg, respectively) determined in Japanese women (7); these are summarized in Table 4. Mean values of digestibility were 96.4 and 95.4% in experiments 1 and 2, respectively. The NPU of egg protein obtained at intake levels of 50, 75 and 100 mg N/kg were 42, 30, and 33 with an energy intake of 33 kcal/kg, and at 30, 50, 75, 100 mg N/kg with an energy intake of 37 kcal/kg were 59, 62, 42 and 46, respectively. The NPU values at the maintenance level of egg protein with an energy intake of 33 and 37 kcal/kg were estimated as 31 and 47, respectively.

DISCUSSION

Inoue *et al.* (3) and Kishi *et al.* (4) studied the effect of energy intake on protein requirement quantitatively. They found that the nitrogen requirement of egg protein decreased with an increase in energy intake; that is, it was 124 mg N/kg at 40 kcal, 90 mg N/kg at 45 kcal/kg, 82 mg N/kg at 48 kcal/kg, and 67 mg N/kg at 57 kcal/kg of energy intake in their male subjects. We investigated the nitrogen requirement of egg protein in women at two levels of energy intake, 33 and

Table 3. Energy and nitrogen intakes, body weight change, urinary creatinine excretion, urinary and fecal nitrogen excretion and nitrogen balance.

Subj. No.	Energy intake (kcal/kg)	Nitrogen intake (mg/kg)	Change in BW ^a (kg)	Urinary creatinine ^b (mg/day)	Urinary N ^c (mg/kg)	Fecal N (mg/kg)	N balance (mg/kg)
Experiment I							
101	30.8	49.4	-2.2	1,077±45	69.2	11.3	-30.1
102	33.4	49.0	-1.1	889±49	49.9	11.6	-10.5
103	30.7	47.0	-2.1	889±79	61.7	10.0	-22.2
104	35.7	50.0	-1.6	1,056±69	53.8	11.3	-13.0
105	36.3	50.0	-0.4	1,050±33	61.3	17.3	-28.7
Mean±SD	33.4±2.6	49.1±1.2	-1.5±0.7	992±95	53.2±7.5	12.3±2.9	-20.9±8.9
106	30.4	76.0	-1.7	1,136±34	89.5	11.3	-22.0
107	29.1	77.0	-1.0	1,082±47	82.9	8.6	-14.7
Mean	29.8	76.5	-1.4	1,109	86.2	10.0	-18.4
108	29.9	99.0	-1.9	1,095±26	96.7	13.1	-9.5
109	32.0	98.0	-3.4	1,105±30	95.9	14.2	-10.9
110	27.7	99.0	-1.5	1,022±30	91.7	9.5	-1.7
111	37.0	103.0	-1.4	882±46	103.7	12.2	-9.6
112	32.1	118.0	-1.1	873±40	109.6	11.7	-0.9
113	37.7	105.0	-0.8	1,056±40	96.8	14.7	-4.3
114	39.2	100.0	-0.8	895±47	101.5	17.2	-12.7
115	35.0	98.0	-2.2	898±61	92.5	20.8	-13.7
Mean±SD	33.8±4.0	102.5±6.7	-1.6±0.9	978±101	98.6±6.0	14.2±3.5	-7.9±5.0

		Experiment 2					
201	34.7	31.7	-1.1	993 ± 71	53.0	11.5	-32.0
202	34.6	32.3	-0.7	908 ± 40	40.9	12.1	-19.5
203	29.6	32.4	+0.5	879 ± 40	40.7	9.8	-17.2
Mean ± SD	33.0 ± 2.9	32.1 ± 0.4	-0.4 ± 0.8	927 ± 59	44.9 ± 7.0	11.1 ± 1.2	-22.9 ± 8.0
204	34.9	50.5	-0.5	963 ± 40	48.6	14.0	-9.5
205	43.0	52.8	-1.8	1,022 ± 20	65.7	9.8	-19.7
206	41.0	50.5	-1.0	831 ± 20	56.5	15.3	-17.5
207	36.6	50.9	+0.1	889 ± 21	44.1	12.9	-3.1
208	35.7	47.1	-0.2	919 ± 39	36.3	12.8	0.9
209	36.7	49.0	-0.8	961 ± 44	41.9	15.2	-6.3
210	40.0	52.0	+0.8	909 ± 67	62.0	14.3	-21.1
211	37.7	52.0	-0.2	985 ± 39	52.1	10.3	-7.3
Mean ± SD	38.2 ± 2.8	50.6 ± 1.8	-0.7 ± 1.4	935 ± 60	50.9 ± 10.1	13.1 ± 2.1	-10.5 ± 8.1
212	35.2	73.5	-1.3	886 ± 31	59.3	13.4	3.6
213	35.4	72.0	-1.8	948 ± 149	79.1	12.1	-16.4
214	39.5	73.8	-0.8	896 ± 42	79.3	10.7	-13.6
215	43.0	74.2	-1.5	1,004 ± 27	76.5	12.6	-13.3
Mean ± SD	38.3 ± 3.7	73.4 ± 1.0	-1.4 ± 0.4	934 ± 54	73.6 ± 9.6	12.2 ± 1.1	-9.9 ± 9.1
216	35.1	98.4	-0.2	954 ± 32	77.1	17.7	6.3
217	42.4	101.6	0	941 ± 32	80.8	11.7	12.4
218	36.0	97.0	-1.0	1,107 ± 57	92.8	14.2	-7.4
Mean ± SD	37.8 ± 4.0	99.0 ± 2.4	-0.4 ± 0.5	1,001 ± 92	83.6 ± 8.2	14.5 ± 3.0	3.8 ± 10.1

^a Difference between the body weight of the last day and mean body weight of the standard period. ^b Mean ± SD of 12 days. ^c Mean of the last 4 days of experimental period.

Table 4. Net protein utilization (NPU) and digestibility of egg protein.

	N intake (mg/kg)	No.	NPU ^a	Digestibility ^b (%)
Experiment 1	50	5	42	96
	75	2	30	100
	100	8	33	96
Experiment 2	30	3	59	97
	50	8	62	94
	75	4	42	97
	100	3	46	96

^a Calculated using values of 32.3 and 10.1 mg/kg for endogenous urinary and fecal nitrogen losses, respectively. ^b Calculated using the value of 10.1 mg/kg for obligatory fecal N loss.

37 kcal/kg. And we found that less egg protein nitrogen was required and that higher *Y* intercept and NPU figures were obtained when more energy was supplied. Comparing our results in females to those in male subjects, the nitrogen requirement of egg protein for females was almost same as for males at an energy intake aimed at weight maintenance during the experimental period, *i.e.*, 37 kcal/kg for women and 45 kcal/kg for men. Furthermore, we calculated the maintenance intake of egg protein per unit basal energy expenditure (basal metabolism, BM) or per unit lean body mass (LBM) from the regression equations obtained from nitrogen intake and balance per these units, respectively. The respective values obtained were 5.93 mg N/kcal BM and 177 mg N/kg LBM in experiment 1 and 4.20 mg N/kcal BM and 132 mg N/kg LBM in experiment 2. To compare the two figures of protein requirement in men and women in detail, further discussion, for example as already referred to above, should be necessary.

In many studies on the minimum need for dietary protein in humans, subjects were provided a higher energy intake than the recommended level in order to increase or maintain body weight during the experimental period. Consequently, a relatively low figure for minimum N was obtained. The requirement of egg protein has been reported to be 4.8 g N/day (68 mg N/kg for 70.1 kg body weight) (10) or 60–94 mg N/kg (11) in Caucasian male subjects. Recently, however, Calloway (6) reported a figure of 89 mg N/kg with an energy intake of 41.2 kcal/kg to be required for body weight maintenance in her male subjects. The energy intakes was 70% higher than the measured basal expenditure. Huang and Lin (12) reported that 98 mg N/kg of egg protein was required with an energy intake of 42 kcal/kg in Chinese male subjects. In these recent studies the investigators determined the optimal energy intake of their subjects. They measured basal expenditure or inquired into the dietary intake and physical activity of their subjects. We investigated the maintenance energy intake for individual subjects by inquiry into

their food consumption prior to the start of the experiment, our subjects being fellow students in our college and trained to give accurate information on food consumption. The mean of their energy intakes was 34.4 ± 3.6 kcal/kg body weight or 1.60 ± 0.18 kcal/kcal BM ($n=33$). With the energy intake provided, body weight was maintained at a constant levels in the standard period but decreased on changing to semi-purified experimental diet in experiment 1. Activities of the subjects did not change throughout the standard and experimental period. The duration of the standard period might be too short or there might be other reasons contributing to the reduction in weight, for example, loss of body water as a result of a sudden decrease in sodium intake from about 12 g/day in the standard diet period to 3 g/day in the semi-purified experimental diet period. This should be further investigated in the future.

In addition, urinary nitrogen excretion (UNE) in female subjects has been reported to vary with the menstrual cycle with a constant intake of energy and protein (13). The menstrual cycle was not taken into account in this study. Larger coefficients of variation of UNE in our subjects than for male subjects (4) might be partly caused by the menstrual cycle, and this variation of UNE with the menstrual cycle should be carefully considered when determining the requirement and utilization of protein in female subjects in the future.

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REFERENCES

- 1) Report of a Joint FAO/WHO Ad Hoc Expert Committee (1973): Energy and Protein Requirements, Wld. Hlth./Org. Techn. Rep. Ser. No. 522.
- 2) Committee on Nutrition (Japan) (1979): Dietary protein allowances, in Recommended Dietary Allowances for Japanese, Ministry of Health and Welfare, Tokyo.
- 3) Inoue, G., Fujita, Y., and Niiyama, Y. (1973): Studies on protein requirements of young men fed egg protein and rice protein with excess and maintenance energy intakes. *J. Nutr.*, **103**, 1673-1687.
- 4) Kishi, K., Miyatani, S., and Inoue, G. (1978): Requirement and utilization of egg protein by Japanese young men with marginal intakes of energy. *J. Nutr.*, **108**, 658-669.
- 5) Garza, C., Scrimshaw, N. S., and Young, V. R. (1976): Human protein requirements: The effect of variation in energy intake within the maintenance range. *Am. J. Clin. Nutr.*, **29**, 280-287.
- 6) Calloway, D. H. (1975): Nitrogen balance of men with marginal intakes of protein and energy. *J. Nutr.*, **105**, 914-923.
- 7) Kaneko, K., and Koike, G. (1983): Obligatory N loss and utilization of egg and rice mixed protein in young Japanese women. *J. Nutr. Sci. Vitaminol.*, **29**, 455-466.
- 8) Koishi, H. (1962): A critical examination on the Folin's method for determination of creatinine concentration in the urine. *Osaka City Med. J.*, **8**, 1-15.
- 9) Brožek, J., Grand, F., Anderson, J. T., and Keys, A. (1963): Densitometric analysis of body composition: Revision of some quantitative assumptions. *Ann. N. Y. Acad. Sci.*, **110**, 113-140.

- 10) Calloway, D. H., and Margen, S. (1971): Variation in endogenous nitrogen excretion and dietary nitrogen utilization as determinants of human protein requirement. *J. Nutr.*, **101**, 205-216.
- 11) Young, V. R., Taylor, Y.S.M., Rand, W. M., and Scrimshaw, N. S. (1973): Protein requirements of man: Efficiency of egg protein utilization at maintenance and sub-maintenance levels in young men. *J. Nutr.*, **103**, 1164-1174.
- 12) Huang, P.-C., and Lin, C. P. (1982): Protein requirements of young Chinese male adults on ordinary Chinese mixed diet and egg diet at ordinary levels of energy intake. *J. Nutr.*, **112**, 897-907.
- 13) Calloway, D. H., and Kurzer, M. S. (1982): Menstrual cycle and protein requirements of women. *J. Nutr.*, **112**, 356-366.