Obligatory N Loss and Utilization of Egg and Rice Mixed Protein in Young Japanese Women

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Summary Obligatory urinary and fecal N losses in young Japanese women were evaluated and the effect of energy intake on the utilization of rice and egg mixed protein was investigated in subjects receiving a low protein diet. Seven female students were given a protein-free diet for ten days. Feces and 24-h urine samples were collected throughout the period and their nitrogen contents were analyzed. Basal metabolism was measured and the lean body mass was calculated from urinary creatinine excretion by the equation of Forbes. The mean weight loss of the 7 subjects during the ten days was 1.5 kg, the mean obligatory urinary N loss was 32.3 ± 5.9 mg/kg body weight, 1.67 ± 0.45 mg/kcal basal metabolism, or 1.68 ± 0.32 g/g creatinine, and the mean obligatory fecal N loss was 10.1 ± 2.0 mg/kg body weight, or 0.51 ± 0.08 mg/kcal basal metabolism. In a second experiment, 17 female subjects were divided into three groups with an energy intake of about 35, 42, 46 kcal/kg BW, respectively. During the 7-day experimental period, they were given a low protein diet containing 250 g of rice and 125 g of whole egg. The nitrogen contents of the 24-h urine samples and feces collected were analyzed and the nitrogen balance was calculated as the difference between N intake and urinary and fecal N excretion. Subjects with an energy intake of 35 kcal/kg BW had a negative N balance, while subjects with an intake of 46 kcal/kg BW achieved an apparent positive N balance. The NPU values of rice and egg mixed protein with energy intake of 35, 42, 46 kcal/kg BW were calculated from the N balance data and the values for obligatory urinary and fecal N losses in the first experiment as 25, 37 and 54, respectively.

Key Words obligatory N loss, protein utilization, women

Protein requirements for Japanese adults (1979)(1) are calculated by the factorial method depending on the estimated obligatory nitrogen loss and the efficiency of utilization of food protein. However, the figures for obligatory N loss, that is the sum of urinary, fecal and integumental losses, and the data on utilization

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of food protein have been estimated in Japanese males only, not in females. In this study, these values were evaluated experimentally in Japanese females.

In the 1973 Report (2), the Joint FAO/WHO Ad Hoc Expert Committee on Energy and Protein Requirements stated that although there was a somewhat lower figure (about 25 mg N/kg BW) for obligatory urinary N loss in women (3-5), it might not be reliable. The Committee assessed the same figure for women as for men from the data by Murlin *et al.* (6) who reported that the difference between men and women was not significant. Obligatory fecal N loss was given as 12 mg/kg BW for men and 9 mg/kg BW for women in the report of FAO/WHO (1973) (2). For Japanese, however, the same figure (12 mg/kg BW) has been applied for both men and women (1).

The effect of the level of energy intake on the efficiency of dietary protein utilization and the N requirement were examined by Inoue *et al.* (7) and their results are supported by those of others (8-11). Recently, Nicol and Phillips (12) reported a considerably higher NPU (net protein utilization) of egg protein in Nigerian men than in Japanese or Caucasian men. This fact suggests that the efficiency of protein utilization also depends on environmental and host factors. Most studies on human protein utilization have been made on male subjects, with only a few studies on Caucasian females and none on Japanese female.

The present experiment was planned to evaluate the obligatory urinary and fecal N losses in young Japanese women. The effect of energy intake on the utilization of rice and egg mixed protein was also investigated in young women receiving a low protein diet. These studies provide fundamental information on the requirement and utilization of protein and energy in women.

EXPERIMENTAL

Obligatory urinary and fecal N losses. Seven female students of 19 to 23 years old (Table 1A) were given a protein-free diet for 10 days (Table 2). Energy intake in each individual was adjusted to meet the level supplied by an ordinary diet, which was recorded for one or two weeks before the experiment. Then the subjects were given conventional foodstuffs containing the determined energy and about 1.25 g of protein per kg body weight for three days. Their body weights did not change significantly during the period on standard diet with an adequate protein intake. So this energy intake was considered as the maintenance level for each person and was maintained throughout the following period on protein-free diet. The mean energy intake of 7 subjects was 33.8 ± 7.4 kcal/kg BW, which was 1.7 times the basal metabolic rate. During the experimental period all subjects lived in a metabolic unit and continued their normal daily activities. They were weighed daily before breakfast after voiding urine. Throughout the period 24-h urine samples were collected, and its total nitrogen was measured by the semimicro-Kjeldahl method, and creatinine by Jaffe's reaction (13). Charcoal fecal marker was given before breakfast on the first experimental day and the day after the experimental period.

Table 1A. Characteristics of young women studied (protein-free diet).

Subj. code	Age (yr)	Height (cm)	Weight ^a (kg)	BM ^b (kcal/kgBW)	LBM ^c (kg)	LBM/BW (%)	Energy intake (kcal/kgBW)
101	19	158	55.9	18.7	38.4	68.7	28.1
102	23	162	63.2	16.4	41.3	65.3	26.8
103	20	162	61.6	16.3	41.8	67.8	26.1
104	20	151	40.1	20.0	28.5	71.1	37.7
105	22	168	75.2	21.0	48.6	64.6	31.9
106	21	157	49.1	21.4	37.0	75.4	42.8
107	21	156	46.3	26.1	27.2	58.7	43.2
Mean	20.9	159.1	55.9	20.0	37.5	67.4	33.8
SD	1.3	5.4	11.9	3.4	7.6	5.3	7.4

^a Mean weight in the preliminary period. ^b Measured during the experimental period.

^c Calculated from the urinary creatinine excretion by the equation of Forbes (1976).

Collected feces were pooled and dried at 100°C and their N contents was determined. Basal metabolism was measured by indirect calorimetry on one day during the experimental period. The lean body mass was calculated from the urinary creatinine excretion by the equation of Forbes and Bruining (14).

Effect of energy intake on the utilization of rice and egg mixed protein. Seventeen female students (Table 1B) were divided into three groups, and given purified, low protein diet with energy intake of about 35, 42 and 46 kcal/kg BW, respectively. Composition of the diet was shown in Table 2. Energy intake was supplied by cornstarch, sugar and shortening. As a protein source, 250 g of rice and 125 g of whole egg were given to all subjects without distinction of differences in stature or energy intake. The protein intake was 31.7 g/day (5,300 mg N/day), of which 15.8 g was supplied from rice and 15.9 g from egg. Energy from lipid was about 25% of the total energy intake, which corresponded to that in the habitual Japanese diet. During the study all subjects lived in a metabolic unit. They were weighed daily before breakfast and continued their normal activities. During the 7-day experimental period, feces and 24-h urine samples were collected for analysis and basal metabolism was measured. The nitrogen balance was calculated from the average urinary N excretion in the last 3 days of the experimental period, the mean fecal N in the 7-day experimental period and the N intake. As integumental and miscellaneous N losses were not taken into account in this study, data are shown as apparent N balances of N intake and fecal N and urinary N loss.

RESULTS

Oligatory urinary and fecal N losses

Basal metabolism measured on one day during the experimental period and

Table 1B. Characteristics of young women studied (rice and egg mixed diet).

Energy intake level (kcal/kg BW)	Subj. code	Age (yr)	Height (cm)	Weight ^a (kg)	BM ^b (kcal/kg BW)	LBM ^c (kg)	LBM/BW (%)
46	201	19	158	46.0	24.9	34.1	74.1
	202	20	151	40.8	27.1	34.3	84.1
	203	20	148	51.0	21.3	33.6	65.9
	Mean	19.7	152.3	45.9	24.4	34.0	74.7
	SD	0.6	5.1	5.1	2.9	0.4	9.1
42	204	22	161	49.9	25.1	34.2	68.5
	205	21	160	45.1	23.9	37.6	83.4
	206	22	153	46.5	19.3	34.2	73.5
	207	21	160	56.3	19.6	42.0	74.6
	208	22	166	52.7	22.8	37.1	70.4
	209	23	155	58.1	21.4	38.0	65.4
	210	21	153	49.1	20.4	29.9	60.9
	211	21	148	41.8	22.3	30.6	73.2
	Mean	21.6	157.0	49.9	21.9	35.5	71.2
	SD	0.7	5.8	5.6	2.1	4.0	6.7
35	212	20	159	52.7	22.7	36.6	69.4
	213	22	153	55.5	20.9	36.5	65.8
	214	23	159	56.0	21.0	41.5	74.1
	215	21	148	48.9	19.6	35.2	72.0
	216	19	158	58.5	18.4	36.3	62.0
	217	23	162	62.1	16.7	34.8	56.0
	Mean	21.3	155.0	55.6	19.9	36.8	66.6
	SD	1.6	5.5	4.6	2.1	2.4	6.7
Overall	Mean	21.2	156.0	51.2	21.6	35.7	70.2
	SD	1.3	5.4	6.0	2.6	3.2	7.3

^a Weight on the first day of the experimental period. ^b and ^c see Table 1A.

lean body mass, calculated from urinary creatinine, are shown in Table 1A. The mean basal metabolism was 20.0 ± 3.4 kcal/kg BW, the mean lean body mass was 37.5 ± 7.6 kg and the percentage of lean body mass to body weight was $67.4 \pm 5.3\%$. Table 3 shows the change in body weight and the obligatory urinary and fecal N losses. In all subjects, body weight decreased significantly during the period on protein-free diet: the mean weight loss of the 7 subjects during 10 days was 1.5 ± 0.8 kg. Urinary total N excretion decreased gradually, reaching a steady state on days 7–10 (Fig. 1). The average values in the last 4 days, from days 7–10 of the experimental period, was regarded as the obligatory urinary N loss for each

Table 2. Composition of experimental diet.^a

Ingredient	Intake (g/day)	
	Protein free diet	Rice and egg mixed diet
Rice	—	250
Whole egg	—	125
Cornstarch	207	30
Sugar	123	65
Shortening	48	21
Agar	5	5
Sodium chloride	3	3
Vitamin mixture ^b	2 tablets	2 tablets
Mineral mixture ^c	4	4

^a Intake is given for a subject receiving, 1,600 kcal/day. Besides this diet, the subjects consumed black tea, green tea and lemon juice. ^b Two tablets of vitamin mixture contained: retinyl palmitate, 2,000 IU; vitamin D₂, 200 IU; vitamin B₁ hydrochloride, 10 mg; riboflavin, 4 mg; pyridoxine hydrochloride, 6 mg; nicotinamide, 50 mg; cyanocobalamine, 10 µg; ascorbic acid, 150 mg; α-tocopherol acetate, 10 mg; and Ca pantothenate, 30 mg. ^c 100 g of mineral mixture contained (in g): CaCO₃, 25.12; KHCO₃, 39.25; MgSO₄·7H₂O, 10.78; FeC₆H₅O₇·6H₂O, 2.91; KH₂PO₄, 21.74; MnSO₄·4H₂O, 0.0369; CuSO₄·5H₂O, 0.1306; KI, 0.0073; and ZnCl₂, 0.0316. This mixture was mainly based upon that of Rose *et al.* (*J. Biol. Chem.*, **182**, 541 (1950)).

Fig. 1. Urinary N loss in 7 young women who consumed protein-free diet.

Table 3. Change in body weight and obligatory urinary and fecal N losses.

Subj. code	Change in BW (kg/10 days)	Urinary creatinine (mg/day)	(mg/kg BW)	Urinary N ^a (mg/kcal BW)	(g/g creatinine)	(mg/kg BW)	Fecal N ^b (mg/kcal BW)	Fecal and urinary N (mg/kg)
101	-1.7	1,066	44.3	2.37	2.32	10.1	0.54	54.4
102	-0.8	1,166	28.3	1.72	1.53	9.4	0.57	37.7
103	-2.2	1,185	33.5	2.06	1.74	7.9	0.48	41.4
104	-0.6	728	30.0	1.50	1.65	7.6	0.38	37.6
105	-2.9	1,416	31.8	1.51	1.69	11.2	0.53	43.0
106	-1.4	1,020	32.0	1.50	1.54	13.2	0.62	45.2
107	-1.1	937	26.0	0.99	1.28	11.5	0.44	37.5
Mean	-1.5	1,074	32.3	1.67	1.68	10.1	0.51	42.4
SD	0.8	216	5.9	0.45	0.32	2.0	0.08	6.1

^a Average for the last 4 days on protein-free diet. ^b Analyzed on pooled samples collected from marker to marker, which were given before breakfast on the first experimental day and the day after the experimental period, respectively.

Table 4. Nitrogen balance and body weight of individual subjects given rice and egg mixed diets with various energy levels.

Subj. code	Energy intake (kcal/kg)	N intake (mg/kg)	Change in BW (kg/7 days)	Fecal ^a N (mg/kg)	Urinary ^b N (mg/kg)	Nitrogen balance (mg/kg)
201	46.7	114.5	+0.3	82.9	17.3	+14.3
202	46.2	130.8	-0.3	102.9	27.5	+0.4
203	44.9	103.6	+0.2	59.8	18.9	+24.9
Mean	45.9	116.3	+0.1	81.9	21.2	+13.2
SD	0.9	13.7	0.3	21.6	5.5	12.3
204	43.5	107.2	-0.9	93.2	21.9	-8.1
205	43.3	118.4	-1.4	124.4	24.8	-30.8
206	43.2	114.5	-1.7	111.7	15.1	-12.3
207	42.9	95.0	-1.0	81.4	16.1	-2.5
208	42.5	101.3	-0.8	85.1	17.5	-1.1
209	41.4	92.0	-0.5	70.4	15.0	+6.6
210	40.3	106.0	-0.7	92.8	16.6	-3.4
211	40.2	124.4	-0.3	108.9	20.6	-5.1
Mean	42.2	107.3	-0.9	96.0	18.4	-7.1
SD	1.3	11.3	0.5	17.8	3.6	11.0
212	37.8	98.9	-1.0	107.4	16.3	-24.8
213	36.9	93.6	-1.0	91.0	19.4	-16.8
214	35.7	93.0	-0.6	104.1	17.6	-28.7
215	34.5	106.0	-0.6	103.4	16.4	-13.8
216	33.6	89.2	-0.4	67.7	14.5	+7.0
217	31.8	83.9	-0.6	74.9	14.6	-5.6
Mean	35.0	94.1	-0.7	91.4	16.5	-13.8
SD	2.2	7.7	0.2	16.7	1.8	13.0

^a As for Table 3. ^b Average for the last 3 days of the experimental period.

individual: the mean obligatory urinary N loss of the 7 subjects was 32.3 ± 5.9 mg/kg BW, 1.67 ± 0.45 mg/kcal basal metabolism or 1.68 ± 0.32 g/g creatinine. The mean obligatory fecal N loss of the 7 subjects was 10.1 ± 2.0 mg/kg BW or 0.51 ± 0.08 mg/kcal basal metabolism.

Effect of energy intake on the utilization of rice and egg mixed protein

The basal metabolism and lean body mass of each subject are shown in Table 1B. Table 4 shows the energy and nitrogen intakes, the change in body weight, the urinary and fecal N excretions and the apparent N balance in each subject. The body weights of subjects receiving 46 kcal/kg BW tended to increase slightly unlike

Fig. 2. Correlation between N balance and energy intake per kg body weight.

Fig. 3. Correlation between N balance and energy intake per basal metabolism.

those of subjects receiving 42 or 35 kcal/kg BW. No significant correlation ($r=0.064$) was found between N balance and N intake, but a significant correlation ($r=0.358$) was found between N balance and energy intake calculated per kg body weight (Fig. 2). Subjects with an energy intake of 35 kcal/kg BW were in negative N balance and subjects with an intake of 46 kcal/kg BW were in apparent positive N balance. The closed circles in Fig. 2 are for two subjects with low energy intake (less than 35 kcal/kg BW) who maintained an N balance of approximately zero. These two subjects scarcely lost any body weight. Figure 3 shows the correlation between

Table 5. Summary of NPU and digestibility of rice and egg mixed diet.

	Energy intake (kcal)	No. of subj.	N intake (mg)	NPU ^a	Digestibility ^b
per kg BW	47-45	3	117	54	91
	44-40	8	109	37	92
	39-35	4	100	25	93
	34-30	2	88	53	95
per kcal BM	>1.8	9	4.89	50	94
	<1.8	8	4.78	33	92
per kg LBM	70-60	5	157	51	93
	59-50	9	148	39	93
	49-40	3	138	24	92

^a Calculated using values of 42.4 mg/kg BW, 2.18 mg/kcal BM and 63.0 mg/kg LBM, respectively, for endogenous N loss. ^b Calculated using the values of 10.1 mg/kg BW, 0.51 mg/kcal BM and 15.1 mg/kg LBM, respectively, for obligatory fecal N loss determined in the experiment on protein-free diet.

energy intake and N balance expressed per kcal basal metabolism. These values, including those two subjects shown by closed circles, showed that the apparent N balance improved with increase in energy intake. When N balance was expressed per kg lean body mass, the same tendency and a higher correlation coefficient ($r=0.705$) was observed. The NPU of rice and egg mixed protein at each level of energy intake was calculated from the N balance data and figures for obligatory urinary and fecal N losses (Table 5). The NPUs obtained at energy intake of 45-47, 40-44 and 35-39 kcal/kg BW were 54, 37 and 25, respectively. However, at a low energy intake of 30-35 kcal/kg BW, a higher NPU value of 53 was observed in two subjects. The average true digestibility of rice and egg mixed protein was calculated as 93%.

DISCUSSION

The obligatory urinary N loss of our 7 subjects, 32.3 ± 5.9 mg/kg BW, agrees well with the figure of 33.3 ± 3.1 mg/kg BW for Japanese men (15). The value for obligatory fecal N loss for Japanese women obtained in this study, 10.1 ± 2.0 mg/kg BW, was a little less than that for men (12.7 mg/kg BW) (15).

Is there any racial difference in obligatory urinary N excretion? Nicol and Phillips (12) summarized the figures determined in male Caucasian, Chinese, Indian and Nigerian subjects and concluded that there was no essential difference in obligatory urinary N loss in different races. However, few studies have been made

on females of different races. Bricker and Smith (4) reported that the obligatory urinary N excretion of 25 young Caucasian women given protein-free diet for 14 days was 25.2 ± 3.3 mg/kg BW. They measured the urinary N excretion in the last 5 days of the period and reported the mean energy intake of their subjects as 45 kcal/kg BW. In the report of FAO/WHO (1973) (2), the committee considered that the Bricker and Smith figure was somewhat low and might not be reliable. Recently, Bodwell *et al.* (16) reported a figure of 30.7 ± 5.8 mg/kg BW for 11 young Caucasian females given a protein-free diet with an energy intake of 41 ± 2.8 kcal/kg BW for 15 days and determined the urinary N excretion in the last 5 days. Jourdan *et al.* (17) reported results on obese Caucasian women with a mean body weight of 116.6 kg when given protein-free diet for 12 days. The obligatory urinary N excretion of these subjects in the last 5 days was 2.36 ± 0.09 g/day. This value per kg body weight (20.6 mg/kg BW) is considerably lower than ours or Bodwell *et al.*, but per g creatinine (1.77 g/g creatinine) it coincided fairly well with the value of Bodwell *et al.* (1.78 g) and with ours (1.68 g). Since urinary creatinine excretion is closely correlated with lean body mass (14), this agreement suggests that obligatory urinary N loss is related to the lean body mass. Thus there is no considerable difference between Japanese and Caucasian females in obligatory urinary N loss per kg lean body mass or per g of urinary creatinine. However, expressing obligatory urinary N loss per basal metabolism, the value for Japanese women (1.67 mg/kcal) was higher than for Caucasian women (1.1–1.3 mg/kcal) (4–6). This could be explained, as one factor, by the lower basal metabolism in our subjects. Somewhat low basal metabolism in Japanese young women is also reported by Kikuchi *et al.* (18). And recently Solomon *et al.* (19) have reported in Caucasian women a low basal metabolism, 20.7 kcal/kg, which was as much as that in our Japanese subjects. So this problem requires further study. On the other hand, obligatory fecal N loss of Japanese women (10.1 ± 2.0 mg/kg BW) was slightly higher than that of Caucasian women (7.7 mg/kg BW) (16).

Inoue *et al.* (7) reported that in their male subjects the NPU of egg protein was dependent on energy intake as well as N intake, and it was suppressed by the deleterious effect of energy restriction and improved by addition of extra energy to diet with an already adequate energy content. The present data on the NPU of rice and egg mixed protein estimated in female subjects with different energy intakes support their results. According to Inoue *et al.*, in men with a maintenance energy intake of 45 kcal/kg BW, the NPU of egg protein was 55 at the N intake level for maintenance of apparent N equilibrium. The present data showed that the NPU of rice and egg mixed protein with energy intake of 40–44 kcal/kg BW was 37. We also observed that two female subjects showed high utilization of absorbed protein (NPU 53) with low energy intake per kg BW. When energy intake was expressed per basal metabolism or per lean body mass, these particular subjects could be included in the group with somewhat excess energy intake. In these two subjects, the estimated basal metabolism was 20–30% lower than the figure calculated from the basal metabolic rate for Japanese women (20) and a percentage of lean body mass

to body weight was lower, that is a larger content of body fat, than average for Japanese young women. Recently, we observed that several young women had a lower basal metabolism, or larger percentage of body fat, like the subjects described above (21). So we conclude that in calculation of the optimum energy intake for women it is not adequate to calculate the value per body weight or per basal metabolism from the basal metabolic rate for Japanese and that the value should be calculated per estimated basal metabolism or per lean body mass. This problem should be studied further to determine the energy and protein requirements of Japanese women more adequately.

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