Table 1. Composition of the 70% Casein Diets

	Experiment 1		Experiment 2	
	+NiA & +B_6 %	−NiA & +B_6 %	+NiA & −B_6 %	−NiA & −B_6 %
Vitamin-free milk casein	70	70	70	70
L-Methionine	0.5	0.5	0.5	0.5
Sucrose	18.5	18.5	18.5	18.5
Corn oil	5	5	5	5
Mineral mixture*	5	5	5	5
Vitamin mixture*	1	0	0	0
NiA-free vitamin mixture*	0	1	0	0
B_6-free vitamin mixture*	0	0	1	0
NiA and B_6-free vitamin mixture*	0	0	0	1

*AIN 93 was used (Reeves, P.G., Components of the AIN-93 diets as improvements in AIN-76A diet. *J. Nutr.*, **127**, 838S–841S (1997)). The diet (+NiA & +B_6) contained 6 mg NiA and 0.8 mg of pyridoxine-HCl per 100 g of diet.

Table 2. Composition of the 40% and 60% Casein Diets

	40% Casein diet		60% Casein diet	
	+NiA %	−NiA %	+NiA %	−NiA %
Vitamin-free milk casein	40	40	60	60
L-Methionine	0.4	0.4	0.6	0.6
Sucrose	48.6	48.6	28.4	28.4
Corn oil	5	5	5	5
Mineral mixture*	5	5	5	5
Vitamin mixture*	1	0	1	0
NiA-free vitamin mixture	0	1	0	1

*AIN 93 was used (Reeves, P.G., Components of the AIN-93 diets as improvements in AIN-76A diet. *J. Nutr.*, **127**, 838S–841S (1997)). The diet (+NiA & +B_6) contained 6 mg NiA and 0.8 mg of pyridoxine-HCl per 100 g of diet.

Experiment 3 (40% and 60% casein diets with or without NiA in the presence of vitamin B_6). The same procedure was performed as with Experiment 1 except for the diets was done. The composition of the diets used in Experiment 3 is shown in Table 2.

Analyses. To measure the conversion ratio of Trp to niacin, the urinary contents of Nam and its metabolites MNA, 2-Py, and 4-Py were measured. This method does not take account of the increased body store of Nam during growth, and the value does not, therefore, represent the net conversion ratio. However, this value is useful for the assessment of the apparent conversion ratio. The conversion ratio was calculated as the sum of the urinary excretions of {Nam + MNA + 2-Py + 4-Py(μmol/day)} × 100/Trp intake during urine collection (μmol/day). The contents of Nam, 2-Py, and 4-Py in the urine were simultaneously measured by the HPLC method of Shibata *et al.*,[3] while the content of MNA in the urine was measured by the HPLC method of Shibata.[6]

The contents of KA[7] and XA[8] in the urine were measured by HPLC.

Trp oxygenase (EC 1.13.11.11),[9] kynureninase (EC 3.7.1.3: the reaction was done in the absence of added pyridoxal 5′-phosphate),[7] kynurenine aminotransferase (EC 2.6.1.7: the reaction was done in the absence of added pyridoxal 5′-phosphate),[10] 3-HA oxygenase (EC 1.13.1.1),[9] kynurenine 3-hydroxylase (EC 1.14.13.9: the reaction was done in the presence of added NADPH),[11] ACMSDase (EC 4.1.1.45),[12] NMN adenylyltransferase (EC 6.3.5.1),[13] Nam methyltransferase (EC 2.1.1.1),[14] 2-Py-forming MNA oxidase (EC 1.2.3.1),[14] and 4-Py-forming MNA oxidase (EC number not identified)[12] were measured as described in the literature.

Results

Experiment 1 (70% casein diets with or without NiA in the presence of vitamin B_6)

Table 3 shows the effects of feeding the 70% casein diet with or without NiA on the body weight gain, food intake, and food efficiency ratio. The food intake was almost the same between the two groups, but the body weight gain was significantly lower in the group fed on the NiA-free diet, as shown in Fig. 1. As a result, the food efficiency ratio was significantly lower in the −NiA group than in the +NiA group. That is, the necessity of niacin itself was observed in the 70% casein diet, even when a sufficient amount of Trp was taken.

The urinary excretion of KA and XA in terms of nmol/g of diet is shown in Table 4. The urinary

Table 3. Effects of Feeding the 70% Casein Diet with or without NiA on Body Weight Gain, Food Intake, and Food Efficiency Ratio (Experiment 1)

	+NiA & +B_6	−NiA & +B_6
Initial body weight (g)	102 ± 2	105 ± 1
Final body weight (g)	205 ± 5	178 ± 4*
Body weight gain (g/19 days)	103 ± 5	73 ± 3*
Food intake (g/19 days)	231 ± 6	223 ± 3
FER[1]	0.45 ± 0.02	0.33 ± 0.01*

[1]FER, Food Efficiency Ratio.
*Statistically significant difference at $p < 0.05$, compared with the +NiA group, as evaluated by Student's t test.

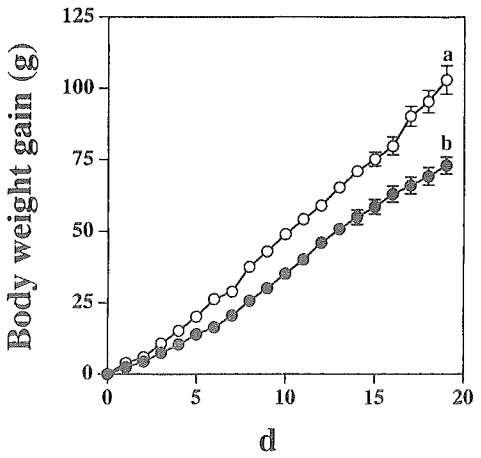

Fig. 1. Effects of Feeding the 70% Casein Diets with or without NiA on Body Weight Gain (Experiment 1).
○, +NiA & +B_6; ●, −NiA & +B_6. Each point represents the mean ± SEM for five rats. Values with different superscript letters are statistically significantly different at $p < 0.05$, as calculated by the Student–Newman–Keuls Multiple Comparisons test.

Table 4. Effects of Feeding the 70% Casein Diets with or without NiA on the Urinary Excretion of KA and XA, Nam and Its Metabolites, the Excretion Ratio of (2-Py + 4-Py)/MNA, and the Conversion Ratio of Trp to Niacin (Experiment 1)

	+NiA & +B_6	−NiA & +B_6
Food intake (g/day)	14.3 ± 0.3	15.0 ± 0.7
KA	198 ± 17	163 ± 15
XA	79 ± 5	62 ± 5
Nam	16 ± 1	3 ± 1*
MNA	87 ± 3	15 ± 3*
2-Py	80 ± 3	11 ± 2*
4-Py	331 ± 9	96 ± 5*
Sum[1]	514 ± 13	125 ± 10*
(2-Py + 4-Py)/MNA	4.7 ± 0.3	7.1 ± 0.8
NiA intake	487 ± 0	0
Trp intake	38783 ± 0	38783 ± 0
Conversion ratio of Trp to Niacin (%)	Not calculated	0.32 ± 0.03

[1]Sum, Nam + MNA + 2-Py + 4-Py.
Values are means ± SEM for five rats, expressed as nmol/g of food, except for (2-Py + 4-Py)/MNA and the conversion ratio of Trp to niacin and means ± SEM for five rats.
*Statistically significant difference at $p < 0.05$, compared with the +NiA group, as evaluated by Student's t test.

excretion of KA and XA was almost the same between the two groups. The urinary excretion of Nam, MNA, 2-Py, 4-Py, and the sum of Nam + MNA + 2-Py + 4-Py in terms of g of diet respectively is also shown in Table 4. The higher values of each of these in the +NiA group than in the −NiA group was attributed to the intake of dietary NiA. The conversion ratio could not be calculated for the group fed on the +NiA diet, but it could be done on the group fed on −NiA diet by comparison with Trp intake during urine collection and the urinary excretion of sum. The value was 0.32 ± 0.03% (mean ± SEM for 5 rats), as shown in Table 4.

Table 5. Effects of Feeding the 70% Casein Diets with or without Niacin on the Enzyme Activities Involved in the Metabolism of Trp to Niacin (Experiment 1)

	+NiA & +B_6	−NiA & +B_6
Trp oxygenase	2.03 ± 0.22	1.77 ± 0.22
Kynureninase	1.57 ± 0.05	1.55 ± 0.04
Kynurenine aminotransferase	1.14 ± 0.10	1.03 ± 0.02
Kynurenine 3-hydroxylase	1.54 ± 0.15	1.96 ± 0.40
3-HA oxygenase	551 ± 35	550 ± 22
ACMSDase	11.3 ± 1.4	12.1 ± 1.1
NMN adenylyltransferase	8.97 ± 0.71	8.31 ± 0.46
NAD^+ synthetase	0.59 ± 0.12	0.61 ± 0.04
Nam methyltransferase	1.85 ± 0.03	1.89 ± 0.04
2-Py-forming MNA oxidase	0.68 ± 0.07	0.71 ± 0.06
4-Py-forming MNA oxidase	1.70 ± 0.08	1.59 ± 0.03

Values are expressed as μmol/h/g of liver and means ± SEM for five rats.

Table 6. Effects of Feeding the Vitamin B_6-Free, and 70% Casein Diets with or without Niacin on Body Weight Gain, Food Intake, and Food Efficiency Ratio (Experiment 2)

	+NiA & −B_6	−NiA & −B_6
Initial body weight (g)	106 ± 1	102 ± 2
Final body weight (g)	139 ± 5	115 ± 4*
Body weight gain (g/19 days)	33 ± 4	13 ± 4*
Food intake (g/19 days)	162 ± 2	141 ± 5*
FER[1]	0.20 ± 0.02	0.09 ± 0.01*

[1]FER, Food Efficiency Ratio.
Values are means ± SEM for five rats, expressed as μmol/h/g of liver and means ± SEM for five rats.
*Statistically significant difference at $p < 0.05$, compared with the +NiA group, as evaluated by Student's t test.

The next step was done to investigate the effects of the 70% casein diet with or without NiA on the enzyme activity of Trp to niacin. As Table 5 shows, none of the enzyme activities showed a difference between the two groups.

Experiment 2 (70% casein diets with or without NiA in the absence of vitamin B_6)

Table 6 shows the effects of feeding the vitamin B_6-free, 70% casein diet with or without NiA on the body weight gain, food intake, and food efficiency ratio. The food intake was significantly lower in the −NiA group than in the +NiA group and the body weight gain was greatly lower in the group fed on the NiA-free diet, as shown in Fig. 2. As a result, the food efficiency ratio was significantly lower in the −NiA group than in the +NiA group. That is, the necessity of niacin itself was also ascertained in the vitamin B_6-free and 70% casein diet.

The urinary excretion of KA and XA in terms of nmol/g of diet is shown in Table 7. The urinary excretion of KA and XA was almost the same between the two groups. But that of XA was much higher in Experiment 2 than in Experiment 1 (Tables 4 and 7).

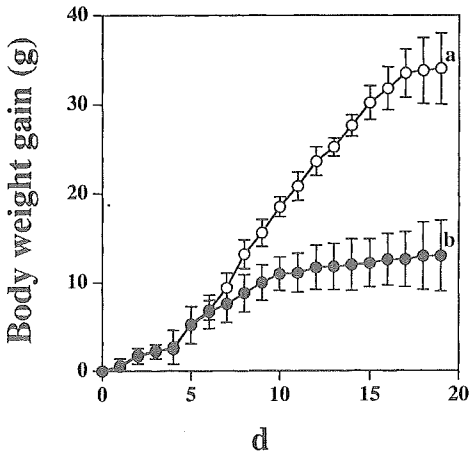

Fig. 2. Effects of Feeding the Vitamin B_6-Free and 70% Casein Diets with or without NiA on Body Weight Gain (Experiment 2).
○, +NiA & −B_6; ●, −NiA & −B_6. Each point represents the mean ± SEM for five rats. Values with different superscript letters are statistically significantly different at $p < 0.05$, as calculated by the Student–Newman–Keuls Multiple Comparisons test.

Table 7. Effects of Feeding the Vitamin B_6-Free, and 70% Casein Diet with or without NiA on the Urinary Excretion of KA and XA, Nam and Its Metabolites, the Excretion Ratio of (2-Py + 4-Py)/MNA, and the Conversion Ratio of Trp to Niacin (Experiment 2)

	+NiA & −B_6	−NiA & −B_6
Food intake (g/day)	7.4 ± 1.3	5.8 ± 1.1
KA	145 ± 5	144 ± 34
XA	1775 ± 173	2100 ± 234
Nam	16 ± 2	11 ± 4
MNA	295 ± 30	65 ± 11*
2-Py	23 ± 3	7 ± 1*
4-Py	137 ± 18	70 ± 6*
Sum[1]	471 ± 48	153 ± 14*
(2-Py + 4-Py)/MNA	0.54 ± 0.04	1.2 ± 0.06*
NiA intake	487 ± 0	0
Trp intake	38783 ± 0	38783 ± 0
Conversion ratio of Trp to Niacin (%)	Not calculated	0.39 ± 0.05

[1]Sum, Nam + MNA + 2-Py + 4-Py.
Values are means ± SEM for five rats, expressed as nmol/g of food, except for (2-Py + 4-Py)/MNA and conversion ratio of Trp to niacin and means ± SEM for five rats.
*Statistically significant difference at $p < 0.05$, compared with the +NiA group, as evaluated by Student's t test.

The abnormal increase in XA means that the rats were in a vitamin B_6-deficient state. The urinary excretion of Nam, MNA, 2-Py, 4-Py, and the sum of Nam + MNA + 2-Py + 4-Py in terms of g of diet respectively is also shown in Table 7. The higher values of each of these in the +NiA group than in the −NiA group was attributed the intake of dietary NiA. The conversion ratio could not be calculated on the group fed on +NiA diet, but it could be done on the group fed on −NiA group by comparison with Trp intake during urine collection and the urinary excretion of sum. The value was 0.39 ± 0.05%, as shown in Table 7.

Table 8. Effects of Feeding Vitamin B_6-Free, and 70% Casein Diet with or without NiA on the Enzyme Activities Involved in the Metabolism of Trp to Niacin (Experiment 2)

	+NiA & −B_6	−NiA & −B_6
Trp oxygenase	1.82 ± 0.09	1.81 ± 0.14
Kynureninase	0.39 ± 0.09	0.32 ± 0.03
Kynurenine aminotransferase	0.38 ± 0.04	0.35 ± 0.04
Kynurenine 3-hydroxylase	3.07 ± 0.17	2.79 ± 0.18
3-HA oxygenase	511 ± 39	565 ± 43
ACMSDase	11.1 ± 1.6	12.6 ± 1.5
NMN adenylyltransferase	8.16 ± 0.27	8.75 ± 0.57
NAD^+ synthetase	0.56 ± 0.07	0.56 ± 0.06
Nam methyltransferase	1.79 ± 0.02	1.82 ± 0.03
2-Py-forming MNA oxidase	0.05 ± 0.02	0.10 ± 0.04
4-Py-forming MNA oxidase	0.18 ± 0.09	0.74 ± 0.10*

Values are expressed as μmol/h/g of liver and means ± SEM for five rats.
* Statistically significant difference at $p < 0.05$, compared with the +NiA group, as evaluated by Student's t test.

The next step was done to investigate the effects of the 70% casein diet with or without NiA on the enzyme activity of Trp to niacin. As Table 8 shows, none of the enzyme activities except for 2-Py- and 4-Py-forming MNA oxidases showed a difference between the two groups.

Experiment 3 (40% and 60% casein diets with or without NiA in the presence of vitamin B_6)

The body weight gain, food intake, and food efficiency ratio in the rats fed the 20%,[1)] 40%, and 60% casein diets with or without NiA are shown in Table 9. These values are almost the same among all of the groups irrespective of dietary protein levels and NiA intake.

Table 10 shows the urinary excretions of Trp–niacin metabolites in the groups of 20%,[1)] 40%, and 60% casein diets with and without NiA. The urinary excretions of KA and XA in terms of nmol/g of diet increased with dietary protein intake irrespective of the presence or absence of vitamin B_6. The higher values of Nam, MNA, 2-Py, and 4-Py in the +NiA group than in the −NiA group between each protein level were attributed the intake of dietary NiA. The conversion ratio could not be calculated on the group fed on +NiA diet, but it could be done on the group fed on −NiA diet by comparison with Trp intake during urine collection and the urinary excretion of sum. The value was 1.90 ± 0.25% for the 20% casein diet,[1)] 1.13 ± 0.07 for the 40% casein diet, and 0.60 ± 0.08% for the 60% casein diet.

Table 11 shows the activities of ACMSDase in the liver. The activities were not different irrespective of presence or absence of vitamin B_6 between the same protein levels, while the activities increased with dietary protein levels.

Table 9. Effects of NiA Addition to the 20%, 40%, and 60% Casein Diets on Body Weight Gain, Food Intake, and Food Efficiency Ratio (Experiment 3)

	20% Casein diet[1]		40% Casein diet		60% Casein diet	
	+NiA	−NiA	+NiA	−NiA	+NiA	−NiA
Initial body weight (g)	103 ± 2	106 ± 2	102 ± 2	102 ± 2	102 ± 1	102 ± 1
Final body weight (g)	216 ± 4	216 ± 4	217 ± 5	223 ± 5	213 ± 4	212 ± 4
Body weight gain (g/19 d)	113 ± 4	110 ± 3	115 ± 4	121 ± 4	111 ± 3	110 ± 4
Food intake (g/19 d)	273 ± 5	287 ± 5	284 ± 5	294 ± 6	262 ± 6	260 ± 3
Food efficiency ratio*	0.41 ± 0.01	0.38 ± 0.01	0.40 ± 0.01	0.41 ± 0.01	0.42 ± 0.01	0.42 ± 0.02

*FER, body weight gain (g/19 d)/food intake (g/19 d).
Each value is the mean ± SEM for five rats.
[1]Data were drawn from reference 1.

Table 10. Effects of Feeding the 20%, 40%, and 60% Casein Diets with or without NiA on the Urinary Excretion of KA and XA, Nam and Its Metabolites, the Excretion Ratio of (2-Py + 4-Py)/MNA, and the Conversion Ratio of Trp to Niacin (Experiment 3)

	20% Casein diet[1]		40% Casein diet		60% Casein diet	
	+NiA	−NiA	+NiA	−NiA	+NiA	−NiA
Food intake (g/day)	16.7 ± 0.6	18.0 ± 0.8	17.9 ± 0.6	18.5 ± 0.7	17.9 ± 0.6	16.5 ± 0.4
KA	35 ± 2.1	40 ± 5.6	105 ± 7.5	126 ± 20.5	156 ± 26.7	191 ± 8.7
XA	28 ± 2.5	36 ± 4.7	55 ± 6.4	51 ± 4.2	67 ± 7.8	81 ± 3.1
Nam	16 ± 0.8	13 ± 1.3	17 ± 1.4	9 ± 0.4	9 ± 1.4	7 ± 0.3
MNA	55 ± 1.1	18 ± 1.8*	57 ± 4.9	22 ± 2.2*	84 ± 8.1	24 ± 1.1*
2-Py	61 ± 2.4	16 ± 2.7*	55 ± 5.3	20 ± 1.9	66 ± 4.1	21 ± 0.9*
4-Py	484 ± 19	161 ± 21*	449 ± 53	199 ± 14*	257 ± 30	149 ± 19*
Sum[1]	616 ± 18	208 ± 27*	578 ± 57	250 ± 17*	416 ± 35	201 ± 20*
(2-Py + 4-Py)/MNA	9.9 ± 0.4	9.8 ± 0.5	8.8 ± 1.5	10.0 ± 1.1	3.8 ± 0.6	7.1 ± 0.7
NiA intake	487 ± 0	0	487 ± 0	0	487 ± 0	0
Trp intake	11081 ± 0	11081 ± 0	22162 ± 0	22162 ± 0	33242 ± 0	33242 ± 0
Conversion ratio of Trp to niacin (%)	Not calculated	1.90 ± 0.25	Not calculated	1.13 ± 0.07	Not calculated	0.60 ± 0.08

[1]Sum, Nam + MNA + 2-Py + 4-Py.
Values are means ± SEM for five rats, expressed as nmol/g of food, except for (2-Py + 4-Py)/MNA and conversion ratio of Trp to niacin and means ± SEM for five rats.
*Statistically significant difference at $p < 0.05$, compared with the respective the +NiA group, as evaluated by Student's t test.
[1]Data were drawn from reference 1.

Table 11. Effects of Feeding the 20%, 40%, and 60% Casein Diets with or without NiA on the ACMSDase Activity in the Liver (Experiment 3)

	20% Casein diet[1]		40% Casein diet		60% Casein diet	
	+NiA	−NiA	+NiA	−NiA	+NiA	−NiA
ACMSDase (μmol/h/g of liver)	2.4 ± 0.6	2.3 ± 0.6	3.81 ± 0.37	3.69 ± 0.57	8.85 ± 0.36	7.90 ± 1.16

[1]Data were drawn from reference 1.

Discussion

Sanada[15] and our group[5,16] have reported that the ACMSDase activity increases with dietary protein levels. We also found that the conversion ratio of Trp to niacin decreases with dietary protein levels.[5,16] Hence we thought that the ACMSDase controls niacin formation to shunt the excessive niacin supply. However, from the present experimental findings, we learned that the view of the Trp–niacin relationship that the ACMSDase controls the niacin formation is not right.

In a previous report,[1] we found that the body weight gains between the rats fed on a diet containing NiA and those fed on the diet minus only NiA were exactly the same. That is, when rats were fed on the 20% casein diet, they do not need niacin. Vitamin B_6 is important in the Trp–niacin metabolism, especially in the metabolism of kynurenine.[17] The body weight gain in young rats was significantly lower in the group fed on the 20% casein diet without vitamin B_6 than in the group fed on the 20% casein diet with vitamin B_6.[1] The lower body weight gain in the vitamin B_6-free group was due to a

deficiency of vitamin B_6.[1] In fact, the urinary excretion of XA, which is an indicator of vitamin B_6 deficiency,[18] was significantly higher in the vitamin B_6-free group than in the vitamin B_6-containing group.[1] However, no necessity of niacin on the 20% casein diet was observed in the diet without vitamin B_6.[1]

Feeding the NiA-free 70% casein diets rats caused a decrease in body weight gain as compared with the 70% casein diets containing NiA (Fig. 1).

In the groups fed on the NiA-free 20%, 40%, and 60% casein diets, the urinary excretion of the sum were 200–250 nmol/g of diet (Table 10), while it was around 130 nmol/g of diet in the groups on the NiA-free 70% casein diet (Tables 4 and 7). These results indicate that the rats fed on the 70% casein diet without NiA were niacin deficient. So the supplementation of NiA to the rats fed on the 70% casein diet caused the growth promoting action. These findings are very curious, since niacin is believed to be supplied from dietary Trp.[19] Under the 20% casein diet conditions, about 2% of Trp is converted to niacin.[1] On the contrary, when rats were fed on 70% protein diets, the conversion ratio was very low, about 0.3% (Table 4), and the rats, therefore, needed niacin for normal growth. The intake of Trp in the group fed on the 70% protein diet increased by 7/2 in comparison with the 20% casein diet, while the conversion decreased by 2/0.3. Therefore, the absolute formation of niacin was about half ($7/2 \times 0.3/2 = 0.525$) that of the rats fed on the 20% casein diet.

The conversion ratio was not affected by the presence or absence of vitamin B_6 on the 70% casein diets (Tables 4 and 7), although it was severely affected by the presence or absence of vitamin B_6 on the 20% casein diets.[1] In the experiment with the 70% casein diets, the urinary excretion of XA was much more increased by feeding the vitamin B_6-free diets (Table 7) than by feeding the B_6-containing diets (Table 4). That is, in Experiment 1, the rats were not vitamin B_6 deficient even when they were fed the 70% casein diet. Therefore, the necessity of niacin in the 70% casein diet was not associated with the nutritional state of vitamin B_6.[20]

The excretion of KA was almost the same among the four groups (Tables 4 and 7). The formations of KA and XA are catalyzed by the same enzyme, kynurenine aminotransferase, which is a PLP-dependent enzyme. This enzyme activity was much lower in the groups fed on the vitamin B_6-free diets (Table 8) than in those fed on the vitamin B_6-containing diets (Table 5). Nevertheless, the flux of Trp to XA increased greatly (Tables 4 and 7). The mechanism in the case of increased XA only can be explained as follows: Kynurenine was more efficiently converted to 3-hydroxykynurenine in the vitamin B_6-deficient rats than in the normal rats because the activity of kynurenine 3-hydroxylase was increased on the vitamin B_6-free diets (Tables 5 and 8), and 3-hydroxykynurenine, therefore, accumulates because the activity of kynureninase, which catalyzes the reaction of 3-hydroxykynurenine to 3-hydroxyanthranilic acid, decreased on the vitamin B_6-free diets (Tables 5 and 8). The accumulated 3-hydroxykynurenine in the group fed on two vitamin B_6-free diets in Experiment 2 was converted to XA by kynurenine aminotransferase. The reason the urinary excretion of KA did not increase might be increased kynurenine 3-hydroxylase.

The side flux of Trp increased in the groups fed on the two vitamin B_6-free diets in experiment 2, but the conversion ratio of Trp to niacin did not change between the groups fed on the diets with or without vitamin B_6 (Tables 4 and 7). This phenomenon has not been explained. The decreased conversion ratio in the high protein diets was due to the increased activity of ACMSDase as compared with that of the 20% protein diets (Tables 5 and 8 and Reference 1). It is a question why the activity of ACMSDase so increased on the high protein diets. High protein diets mean low carbohydrate diets, so that under the conditions, amino acids can be catabolized into energy formation pathways but not into protein synthesis and other biofactors. But in the present experiments it was clearly shown that the conversion ratio of Trp to niacin is subjected to the reaction α-amino-β-carboxymuconate-ε-semialdehyde $\rightarrow$ α-amino muconate-ε-semialdehyde, which is catalyzed by ACMSDase, and α-aminomuconate-ε-semialdehyde is then catabolized into acetyl-CoA, but not into niacin. On the contrary, the reaction α-amino-β-carboxymuconate-ε-semialdehyde $\rightarrow$ quinolinic acid is non-enzymatic, and quinolinic acid is then metabolized into niacin. Accordingly, quinolinic acid formation from Trp is subjected to the activity of ACMSDase. The administration of an inhibitor of ACMSDase causes the greatly increased conversion ratio of Trp to niacin.[21]

In conclusion, we found that rats need dietary niacin when they are fed a 70% casein diet for maximum growth, while they do not need it when they are fed 20%, 40%, or 60% diets. This phenomenon is attributed to changes in the Trp–niacin conversion ratio due to the amount of protein intake. Therefore, when evaluating niacin requirements or status, protein intake must be considered.

Acknowledgment

This investigation was supported by a Grant-in-Aid for Scientific Research (Comprehensive Research on Cardiovascular Diseases), from The Ministry of Health, Labor and Welfare.

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日本家政学会誌 Vol. 56 No. 4 265～272 (2005)

ノート

カツオ由来ナイアシン高濃度含有パウダーのナイアシンとしての生物有効性

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原稿受付平成16年11月2日；原稿受理平成17年2月16日

Bioavailability of Nicotinamide-Rich Powder Obtained from Bonito as a Niacin Source in Humans and Rats

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The bioavailability of niacin in bonito powder high in nicotinamide content was investigated in female college students. They were given a semi-purified diet based on the Japanese Dietary Reference Intakes for 4 days. On days 1, 2, they were given only the required diet for obtaining controlled urinary excretion of the nicotinamide metabolites. On day 3, 15 g of the bonito powder (5 g of the powder after each meal) containing 51 mg of nicotinamide was administered. The urinary excretion of the nicotinamide metabolites was significantly increased, with 52% being excreted on day 3. On day 4, they were given only the required diet, however, the urinary excretion of the nicotinamide metabolites was still higher than the scores for days 1 and 2. These findings indicate that the bioavailability of nicotinamide in the bonito powder was high and provided an excellent source of niacin.

(Received November 2, 2004; Accepted in revised form February 16, 2005)

Keywords: bonito カツオ, niacin ナイアシン, bioavailability 生物有効性, nicotinamide-rich powder ナイアシン高濃度含有パウダー, vitamin ビタミン.

1. 緒 言

ナイアシンにはニコチン酸とニコチンアミドの他に糖やタンパク質と結合した結合型ナイアシンも存在する．結合型ナイアシンは穀類中に多く存在し，消化されにくいため，哺乳動物には利用されにくい[1]．したがって，ある食品をナイアシンの供給源として評価するためには，化学的な方法によって測定されたナイアシン量のみならず，生物有効性も重要な因子となる．

世界的にみると，ビタミンの欠乏症で最も頻繁にみられるのは，ペラグラである[2]．米国では，20世紀初頭に大発生し，公衆衛生上の大きな問題となり[3～6]，結果的に有効物質であるナイアシンの発見[7]に繋がったという経緯がある．Millerは[8]，1978年に米国におけるペラグラによる死者をまとめている．日本ではペラグラの発生と時を同じくしてビタミンB_1欠乏である脚気が流行したが[9]，ペラグラの発生は報告されていない．これは，ナイアシンの豊富な魚を食していたことと無関係ではないと思われる．カツオは特にナイアシン含量の高い魚であるが，保存の点で扱いにくい魚であった．しかし，最近，我々は，新しい手法で，保存性の優れたカツオ由来の高濃度ナイアシン含有パウダーを開発することに成功した（後述の実験方法の項を参照）．そこで，本実験では，ヒトを用いて，ナイアシン高含有カツオパウダー（カツオパウダー）に

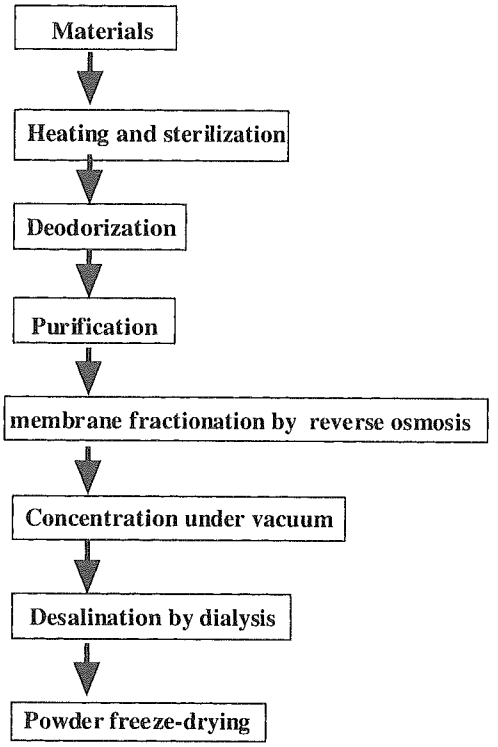

Fig. 1. Flow chart for making the bonito powder high in nicotinamide content

Table 1. Major components of the bonito power high in nicotinamide content

	%
Water	0.79
Total nitrogen	14.37
Lipid	0.12
Ash	14.18
NaCl	0.08

含まれるナイアシンの生物有効性について検討した．

さらに，ニコチンアミドには糖尿病を予防する作用があること（ニコチン酸にはこの作用はない）が実験用小動物で証明されている[10]．そこで，本実験では，カツオパウダーをラットに摂取させ，STZ 誘発性糖尿病を予防できるか否かについても検討した．

Table 2. Amino acid composition of the bonito powder high in nicotinamide content

Amino acid	mg/100 g
Phosphoserine	74
Taurine	531
Aspartic acid	318
Hydroxyproline	140
Threonine	409
Serine	498
Glutamic acid	942
Sarcosine (N-methylglycine)	348
α-Aminoadipic acid	0
Proline	735
Glycine	2,425
Alanine	1,604
α-Aminobutyric acid	15
Valine	561
Cystine	19
Methionine	369
Isoleucine	374
Leucine	831
Thyrosine	385
Phenylalanine	469
β-Alanine	1,177
γ-Aminobutyric acid	15
Histidine	19,593
1-Methylhistidine	2,480
Hydroxylysine	30
Ornithine	92
Lysine	1,353
Arginine	5,234
Total	41,021

2. 実験方法

(1) ナイアシン高含有カツオパウダー製造方法の概略

カツオ節製造時に副産する煮汁を出発物質として，Fig. 1 に示した製造フローに従って製造した．Table 1 に主な成分含量を，Table 2 にアミノ酸含量を示した．

(2) 第一実験（ヒトを用いた生物有効性を求めるための実験）

1) 被 験 者

21～23 歳の健康な女性 8 名（身長，159.5±1.2 cm；体重，53.0±0.8 kg）を対象とした．厚生労働省が示

Table 3. Composition of the control diet

	(g/day)	Remarks
Vitamin-free milk casein	22.6	The casein contained 87.5% protein, so the net protein amount was 19.8 g. The tryptophan content was 1.3%, so 257 mg of tryptophan was supplied.
Gluten	43.2	The gluten contained 81.6% protein, so the net protein amount was 35.3 g. The tryptophan content was 1.1%, so 388 mg of tryptophan was supplied.
		Total protein=55 g
		Total tryptophan=645 mg
Cornstarch	250	
Sucrose	50	**Total carbohydrate=300 g**
Fat		**Total fat=46 g**
Soybean oil	10.6	
Rapeseed oil	16.0	
Coconut oil	6.1	
Lard	13.3	
Dietary fiber		Soluble dietary fiber, Fibersol, was obtained from Matsutani Chemical
Soluble	3.6	Industry Co. Ltd. (Osaka, Japan), and insoluble dietary fiber, Ramie, was ob-
Insoluble	14.4	tained from Tosco Co., Ltd. (Tokyo, Japan).
Mineral mixture	13.8	The composition is shown below.
Total amount (g)	425.6	
Total energy (kcal)	1,834	

For breakfast and supper, 128 g of the diet was added to 90 ml of water, mixed well, and baked for 9 min at 250℃. The weight of the baked meal was *ca.* 175 g (170–180 g). The meal and 0.3 g of the vitamin mixture (composition shown below) were given to the subjects. For lunch, 170 g of the diet was added to 120 ml of water, mixed well, and was baked for 10 min at 250℃. The weight of the baked meal was *ca.* 233 g (225–240 g). The meal and 0.4 g of the vitamin mixture (composition shown below) were given to the subjects. Composition of the mineral mixture: 1,100 mg of $CaHPO_4 \cdot 2H_2O$, 860 mg of $CaCO_3$, 2,200 mg of KH_2PO_4, 3,500 mg of $KHCO_3$, 2,100 mg of $MgCl_2 \cdot 2H_2O$, 60 mg of $FeSO_4 \cdot 5H_2O$, 13 mg of $MnSO_4 \cdot 5H_2O$, 19 mg of $ZnCl_2$, 6.3 mg of $CuSO_4 \cdot 5H_2O$, 0.2 mg of KI, and 4,000 mg of NaCl. Composition of the vitamin mixture: 3.6 mg (1,800 IU) of retinal acetate reagent (500,000 IU/g), 2.5 μg of cholecalciferol, 5.1 mg of *dl-α*-tocopherol (4.6 mg was supplied from oils), 16 μg of phylloquinone (39 μg was supplied from oils), 0.9 mg of thiamin-HCl, 1.0 mg of riboflavin, 1.5 mg of pyridoxine-HCl, 2.4 μg of cyanocobalamin, 5.5 mg of calcium panthotenate, 200 μg of pteroylmonoglutamic acid, 30 μg of D(+)-biotin, and 100 mg of ascorbic acid, made up to 1 g with sucrose.

す食事摂取基準[11]（生活活動強度Ⅱの18～29歳女性，約1,800 kcal/day，タンパク質55 g/day，炭水化物300 g/day，脂質46 g/day）を満たす食事（Table 3）を4日間与えた．食事中にはビタミン体のナイアシンは0であるが，トリプトファンが645 mg含まれ，この量はナイアシン当量として，約11 mgである．被験者は，一定の生活スケジュール（起床，6時；朝食，7時30分；昼食，12時30分；夕食，18時30分；就寝，11時）に従って行動させた．飲料については，市販の水を自由摂取させた．実験開始日をday 1とした．day 3に5 gのカツオパウダー（ナイアシン17 mgを含む）を朝食後，昼食後，夕食後の計3回，食事終了後，別途摂取させた．カツオパウダー由来のナイアシン摂取量は51 mg，食事由来のナイアシン当量摂取量は11 mgであった．起床後2回目から翌日起床直後までの尿を24時間尿とし，実験期間中4日分の24時間尿を採集した．

2) 尿中のニコチンアミド代謝産物の測定

N^1-メチルニコチンアミド（MNA）については，尿中のMNAをアセトフェノンと縮合させることにより蛍光物質に変換し，HPLCを用いて定量した[12]．

N^1-メチル-2-ピリドン-5-カルボキサミド（2-Py）およびN^1-メチル-4-ピリドン-3-カルボキサミド（4-Py）については，尿に炭酸カリウムを飽和量加えた後，ジエチルエーテルで抽出し，乾固させた抽出物を水に溶解させた．HPLCを用いてこの溶解物を定量し

Table 4. Composition of the diets

	Control diet (NiA-free 22% casein) (%)	Test diet (control diet+bonito powder) (%)
Vitamin-free casein	22	20
L-Methionine	0.2	0.2
Gelatinized cornstarch	44.5	42.5
Sucrose	22.3	21.3
Corn oil	5	5
Mineral mixture	5	5
Vitamin mixture*	1	1
Bonito powder	0	5

* The vitamin mixture was niacin-free.

た[13].

3) カツオパウダー中のニコチンアミド量の測定

カツオパウダー1gに水1lを加え，室温で10分間撹拌した溶液をニコチンアミド測定用試料とした．試料に炭酸カリウムを飽和量加えた後，ジエチルエーテルで抽出し，乾固させた抽出物を水に溶解させた．HPLCを用いて定量を行った[13]．その結果，本実験に使用したカツオパウダー中のニコチンアミド量は3.4 mg/gであった．この含量（3.4 mg/g）は五訂に本食品標準成分表に記載されているカツオ節中のナイアシン含量の約7.5倍であった．

(3) 第二実験（STZ-誘発糖尿病ラットに対する影響）

本実験は滋賀県立大学動物実験委員会の承認を受けたものである．

1) 動物の飼育方法

5週齢のWistar系雄ラット20匹を日本クレア㈱より購入し，体重が均等になるよう10匹ずつ2群に分けた．試験食群として，20%カゼイン食に5%となるようカツオパウダー（ナイアシン含量340 mg/100 gカツオパウダー）を添加したカツオ食群（ナイアシン含量；17 mg/100 g飼料）を設定した（Table 4）．対照群として，カツオ食中のアミノ酸含量と等しくなるよう22%カゼイン食群を設定した（Table 4）．ラットは1匹ずつ金網ケージにて29日間飼育し，飼料と水は自由摂取とした．動物室の温度は20℃前後，湿度は50%前後，明暗サイクルは6時から18時を明，18時から6時までを暗とした．飼育開始日をday 0とした．

Day 8の午前9時に対照群，試験食群をそれぞれ5匹ずつ，対照食-生理食塩水群及び対照食-STZ群，試験食-生理食塩水群及び試験食-STZ群の4群に分けた．対照食-STZ群及び試験食-STZ群には，生理食塩水0.5 mlに70 mg/kg body weightとなるように溶解させたストレプトゾトシン（STZ）を腹腔内注射した．また，対照食-生理食塩水群及び試験食-生理食塩水群には生理食塩水0.5 mlを腹腔内注射した．

Day 28の9時より6時間絶食し，15時に尾静脈から採血した．小型血糖測定機グルテストエース（㈱三和化学研究所）を用いて空腹時血糖を測定した．

Day 29にラットを断頭屠殺し，直ちに頸動脈血を採取し，血中NAD含量及び血中NADP含量を測定した．また，肝臓を摘出し，肝NAD含量，肝NADP含量及び肝α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase（ACMSD）活性を測定した[14]．

2) NAD・NADPの定量方法[15)-18)]

i) 測定機器

測定に使用したマイクロプレート用吸光測定装置はLabsystemsのMultiskan Ascent（Thermo Bioanalysis Company, FIN-00811 Helsinki, Finland. 輸入元：サーモバイオアナリシスジャパン株式会社．販売元：大日本製薬株式会社 ラボラトリープロダクツ部）を使用し，570 nmのフィルターを使用した．

マイクロプレートタイターは住友ベークライト㈱のELISA用プレートSを使用した．

ii) 抽出方法

血液及び肝臓からのNAD・NADP測定用試料の作製方法はShibataら[15]が開発した0.1 Mニコチンアミドを含む50 mMリン酸カリウム緩衝液，pH 6.0を用

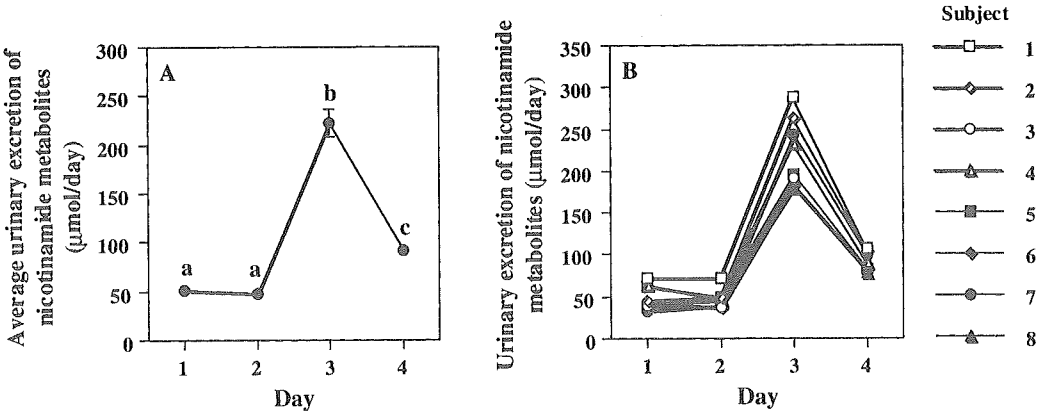

Fig. 2. Average urinary excretion (A) and individual excretion (B) of nicotinamide metabolites (Experiment 1)

Each value is the mean±SEM ($n=8$) (A). A different superscript letter means significant difference at $p<0.05$ as determined by paired ANOVA with post hoc testing with Tukey's multiple-comparison test.

いる熱抽出法で行った.

iii) 測定方法

NAD・NADP の測定方法は，柴田ら[15)~18)]が報告した酵素サイクリング法に従って行った.

3. 結　果

(1) 第一実験

ヒトにおいてナイアシンは MNA，2-Py，4-Py のいずれかの化合物として尿中に排泄されることから，MNA，2-Py，4-Py の合計を総ニコチンアミド代謝産物量とし，カツオパウダーに含まれるナイアシンがどの程度利用されるのか調べた．規定の食事を与えると尿中の総ニコチンアミド代謝産物量は day 1 では 53±5 μmol/day，day 2 では 47±4 μmol/day であったが，カツオパウダーを服用した day 3 では 222±14 μmol/day に増加した（Fig. 2A）．翌日の day 4 でも 93±4 μmol/day と，投与前の値よりも高い値を示した．この現象は被験者 8 名全員に共通していた（Fig. 2B）．Day 1 および day 2 の平均尿中総ニコチンアミド代謝産物量は 49±4 μmol/day（day 1 および day 2 のナイアシン当量摂取量は約 11 mg，すなわち約 90 μmol である．したがって，摂取量に対するニコチンアミド異化代謝産物の排泄量比は 54%である）であったことから，カツオパウダーの摂取により増大した量は 217 μmol/day（222+93−49−49=）と推定した．この値はカツオパウダー 15 g 中に含まれるナイアシン 51 mg（418 μmol）の 52%であった.

(2) 第二実験

1) 飼料摂取量と体重増加量

飼料摂取量と体重の変化量を各々 Fig. 3A と Fig. 3B に示した．Day 8 までの予備飼育期間において，対照食群と試験食群との間に飼料摂取量及び体重増加量の違いは認められなかった．対照食-STZ 群及び試験食-STZ 群の飼料摂取量は STZ 投与直後に減少したが，day 11 より増加しはじめ，積算飼料摂取量は対照食-生理食塩水群及び試験食-生理食塩水群の約 1.5 倍となった．しかし，対照食-STZ 群及び試験食-STZ 群の体重増加量は，対照食-生理食塩水群及び試験食-生理食塩水群より低く，30~40%であった．飼料摂取量及び体重増加量において，対照食-生理食塩水群と試験食-生理食塩水群間に，また対照食-STZ 群及び試験食-STZ 群間に有意な差異は認められなかった.

2) 血 糖 値

対照食-STZ 群及び試験食-STZ 群の血糖値は対照食-生理食塩水群及び試験食-生理食塩水群間に比べ，著しく高い値となった（Table 5）．また，対照食-STZ 群と試験食-STZ 群間の血糖値に差は認められなかった.

3) 血中 NAD・NADP 含量

ナイアシン摂取量が必要量を満たさない場合には血中 NAD 含量は低くなり，必要量に達すると飽和値を示すことから[19)]，血中 NAD 含量はナイアシン栄養状態を反映する．対照食-生理食塩水群，試験食-生理食

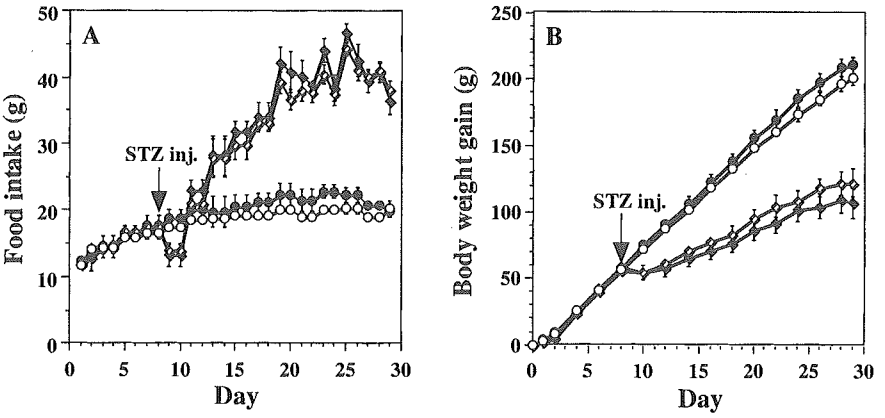

Fig. 3. Food Intake (A) and Body Weight Gain (B) (Experiment 2)

○, Control diet with saline injection; ◇, Control diet with STZ injection; ●, Test diet with saline injection; ◆, Test diet with STZ injection. Each value is the mean±SEM (n=5).

Table 5. Effects of the bonito powder intake and STZ injection on the body weight, food intake, liver weight, blood glucose level, and niacin metabolism

	Control diet saline injection	Control diet STZ injection	Test diet saline injection	Test diet STZ injection
Initial body weight (g)	111.9±1.3	111.9±1.3	112.0±1.4	112.3±1.6
Body weight at day 8 (g)	168.5±1.5	170.6±2.2	170.1±3.7	169.1±5.7
Final body weight (g)	313.6±5.6^{a}	234.4±10.2^{b}	323.0±7.0^{a}	219.4±12.8^{b}
Food intake* (g/21 days)	403.6±4.0^{a}	686.1±19.9^{b}	440.5±14.4^{a}	711.4±28.9^{b}
Body weight gain* (g/21 days)	145.1±4.8^{a}	63.8±10.9^{b}	153.0±4.9^{a}	50.3±9.3^{b}
Food efficiency ratio**	0.359±0.008^{a}	0.094±0.014^{b}	0.348±0.006^{a}	0.070±0.012^{b}
Liver weight (g)	15.1±0.4	12.7±0.8	14.4±0.6	12.2±0.6
ACMSD activity (μmol/h/g of liver)	2.1±0.4^{a}	23.6±3.0^{b}	2.2±0.5^{a}	29.5±2.2^{b}
Blood glucose level (mg/dl)	94±3^{a}	433±11^{b}	99±3^{a}	482±40^{b}
Blood NAD level (nmol/ml)	80.9±2.1^{a}	66.0±2.3^{b}	84.7±2.9^{a}	85.5±5.7^{a}
Blood NADP level (nmol/ml)	14.3±0.6^{ab}	15.9±0.9^{bc}	15.5±0.7^{bc}	18.3±0.4^{c}

*Data were calculated as total from day 8 to day 29. **FER=body weight gain (g/21 days)/food intake (g/21 days). Each values is the mean±SEM (n=4-5). Values with different superscript letters are statistically different at $p<0.05$ by Tukey's multiple-comparison test.

塩水群及び試験食-STZ群間の血中NAD含量に差異は認められなかった（Table 5）．対照食-STZ群の血中NAD含量は他の3群に比べ，有意に低い値を示した．

一方，血中NADP含量はナイアシン摂取量の多少に関わらず容易に変動しないことが報告されている[20]．しかしながら，今回の実験においては，試験食-STZ群の血中NADP含量は対照食-生理食塩水群よりも高い値を示した．なお，他の群とは有意な差異は認められなかったことから，やはりNADP含量は変動しにくいものと思われた．

4） 肝ACMSD活性

STZ誘発糖尿ラットでは肝ACMSD活性が著しく高くなることが報告されている[14]．今回の実験においても，対照食-STZ群及び試験食-STZ群の肝ACMSD活性は対照食-生理食塩水群及び試験食-生理食塩水群間の約12倍の値を示した（Table 5）．

4. 考　察

体内のナイアシンプールが飽和に達すると，余剰のナイアシンはニコチンアミド代謝産物として尿に排泄される[19]．したがって，尿中の総ニコチンアミド代謝産物量を指標として，食品中のナイアシンの生物有効性を調べることが可能となる．本実験において，女子学生にナイアシンを高濃度で含むカツオパウダーを投与したところ，投与量の52%がニコチンアミド代謝産物として尿中に排泄された．一方，女子学生にニコチンアミド標品50 mgをそれぞれ朝食後，昼食後，夕食後の計3回，総量で150 mg服用させると，ニコチンアミド服用量の64%が投与日の24時間尿中に排泄されることが報告されている[20]．また，ニコチンアミド75 mgを含む総合ビタミン剤を男子学生に服用させると，ニコチンアミド服用量の55%が投与日の24時間尿中に排泄されることが報告されている[21]．これらの既報の報告は[20][21]，本研究のニコチンアミド51 mgの負荷に比べ多い摂取量であるが，カツオパウダー中のニコチンアミド代謝産物排泄率はこれらの報告と近い値であった．したがって，カツオパウダー中のナイアシンはサプリメントやビタミン剤として使用されているニコチンアミド標品と同等の生物有効性を持つと考えられる．

自己免疫的機序により膵β細胞が傷害を受けると，インスリン依存型糖尿病を発症する．そのモデル動物を作製するために，膵β細胞に対して特異的に毒性を示すストレプトゾトシン（STZ）が用いられている[10]．一方，STZ投与時に大量のニコチンアミドを同時投与することにより，STZ誘発性糖尿病を予防することが報告されている[22]．そこで，カツオパウダー投与がどの程度STZ-誘発糖尿ラットの予防に有効であるか否かを調べてみた．その結果，STZ誘発糖尿ラットの血糖値及び体重増加量に対し，カツオパウダー摂取による予防，改善は見られなかった．STZ誘発性糖尿病の予防効果を示したニコチンアミドの投与量は500 mg/kg body weightと大量であり[22]，β細胞内のNAD濃度は一過性に高濃度になることが推察される．本実験においてSTZ投与までのニコチンアミド摂取量は約20 mg/kg body weightであり，また経口摂取したことと併せ，ニコチンアミド腹腔内投与時に比べβ細胞内NAD濃度が低いためにカツオパウダー摂取による糖尿病予防・改善効果が見られなかったことが推察される．なお，今回用いたカツオパウダー中にはニコチン酸は含まれておらず，ニコチンアミドのみが含まれている．

トリプトファン-NAD生合成経路において，α-amino-β-carboxymuconate-ε-semialdehydeはACMSDによって代謝されるとアセチルCoAにまで代謝され，さもなくば自己環状化してキノリン酸になることによってNADにまで代謝される．STZ誘発糖尿ラットではACMSD活性が著しく高くなるため，トリプトファンからのNAD生合成量が減少し，ナイアシン栄養状態が悪くなる．対照食-STZ群の血中NAD含量は対照食-生理食塩水群に比べ有意に低く，ナイアシン栄養状態の悪化を示している．しかし，試験食-STZ群はカツオパウダーを摂取したため，血中NAD含量は対照食-生理食塩水群及び試験食-生理食塩水群と同じレベルを維持しており，ナイアシン栄養状態の悪化を防いでいる．この結果から，カツオパウダーはナイアシン栄養状態の悪化を予防・改善するためのナイアシン供給源となりうる可能性がある．

結論として，我々が前に報告した小麦ふすま[23]とコーヒー抽出液乾固物（インスタントコーヒーパウダー）[24]と同様に，今回作製したカツオ由来のパウダーは，ナイアシンを生物有効性の高い遊離型のニコチンアミドとして含有していることが示唆された．また，カツオパウダーはカツオ節と同様に味質的にも優れ，かつ保存性にも優れていることから，通常食品として，さらにはトウモロコシ多食地域のペラグラ予防食品としても有用であると考えられる．

5. 要　約

(1) ヒトを用いて，カツオ由来ナイアシン高濃度含有パウダー（カツオパウダー）中のナイアシンの生物有効性について検討した．21～23歳の健康な女子学生8名を対象として，食事摂取基準を満たす食事を2日間与えた後，ナイアシン51 mgを含むカツオパウダー15 gを摂取させた．カツオパウダーを摂取した日に，摂取カツオパウダー中のナイアシンの52%がニコチンアミド代謝産物として尿中に排泄された．カツオパウダー中のナイアシンはニコチンアミド標品に近い，高い生物有効性を持つことが示唆された．

(2) カツオパウダーがストレプトゾトシン（STZ）誘発性糖尿病の予防・改善効果を有するか検討した．5週齢のWistar系雄ラットにカツオパウダー添加食を8日間与えた後，STZ 70 mg/kg body weightを腹腔内注射し，さらにカツオパウダー添加食を21日間与えた．カツオパウダー摂取によるSTZ誘発性の糖

尿病の予防・改善は見られなかった．しかし，STZ誘発性糖尿病によるナイアシン栄養状態の悪化を防止した．

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ノ ー ト

トリプトファン-ナイアシン代謝に関与する酵素活性から推定したラット乳仔のトリプトファン-ナイアシン転換率

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Vitamins(Japan), **79**(8), 391-393(2005)

Estimated Conversion Ratio of Tryptophan-Niacin in Nursing Rats Using the Enzyme Activities Involved in the Tryptophan-Niacin Metabolism

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Although the conversion ratio of tryptophan-niacin has been reported to be 1/60 by the weight basis in human adults, there is no data on nursing infants of humans. It is estimated at 0 in 0-5 month-old nursing infants of humans and at 1/120 in 6-11month-old of humans from extrapolating data obtained in the weaning rats and the adult rats. Then, in order to estimate the conversion ratio of tryptophan-niacin in nursing infants of humans, the rats from newborn to before weaning were used; the enzyme activities involved in the tryptophan-niacin metabolism were measured and compared with those of the adult rats. Consequently, tryptophan-2,3-dioxygenase (TDO) activity which is the first enzyme in the tryptophan-niacin conversion pathway was very weak in the 1st day immediately after birth, and also in the 7th day. These results mean that niacin is hardly biosynthesized from tryptophan in the early stages of nursing infants in rats. Moreover, since the TDO activity on the 14th and 21st increased with age, when applying these data to humans, it was thought in the latter period of nursing infants of humans i.e., 6-11 month-old, that this conversion pathway begins to operate and it is about 1/120 which is the half of the human adults.

Keywords: *nursing infant, weaning rat, tryptophan, enzyme activity, metabolism*

(*Received December 22, 2004*)

緒 言

乳児(0～11 カ月)の水溶性ビタミン必要量の算定精度が低いことが指摘されている[1]．乳児の水溶性ビタミン必要量を求めるための根本的な考え方は，「乳児(0～5 カ月)は，母乳を適当量摂取している限り，健常に発育する．」[1]というものである．また，6 カ月から 11 カ月の乳児は離乳食を摂るようになるので，必要量を求めるのは複雑であるため，精度の高いデータを得ることが困難であり，我々が調べた限り報告は認められなかった．現在の必要量の求め方の考え方は，「0～5 カ月児の必要量からの外挿値と成人の必要量からの外挿値を，平均化したものが適当であろう」[1]である．具体的に述べれば，乳児(0～5

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カ月)の必要量に体表面積比，(6～11カ月の基準体重/0～5カ月の基準体重)$^{0.75}$をかけた数値と成人(18～29歳)の必要量に，{(6～11カ月)の基準体重/成人(18～29歳)の基準体重}$^{0.75}$×(1+成長因子の0.3)}をかけた数値の，二つの値の平均値である[1]．これらの6～11カ月の乳児の必要量の外挿値は，若年成人から外挿した値よりも乳児から外挿した値の方がかなり低くなる．単位重量当たりの代謝回転は乳児の方が若年成人よりも高いと思われるのに，なぜであろうかという疑問を解決するために，このシリーズの研究を開始した．

ナイアシンは必須アミノ酸のトリプトファンから体内で合成される．その量は，成人では，重量比で1/60である[2]．ところが，この比率は年齢によって変動し，「第六次改定日本人の栄養所要量—食事摂取基準—」では，離乳直後のラットと成熟期のラットにおける本転換経路に関与する酵素活性の比較から，0～5カ月の乳児では0，6～11カ月の乳児では1/120としている[3]．離乳前のデータに関しては，Greengard と Dewey[4]が，ラットの乳仔についてトリプトファン2,3-ジオキシゲナーゼ(TDO)活性について調べた成績が報告されている．出生12日まではTDOが全く検出されなかったが，離乳期の21日ではすでに成熟ラットの値と同じ程度の活性が検出されたことを報告している．しかしながら，他のトリプトファン-ナイアシン転換経路の酵素活性に関する報告はない．そこで，今回は，乳児期のトリプトファン-ナイアシン転換率の数値の精度を高めるために，ラット新生仔におけるトリプトファン-ナイアシン転換率に関与する酵素活性の測定を行ったので報告する．

実　験　方　法

1．ラットの飼育方法

妊娠20日目のWistar系，雌ラットを日本クレア(株)より購入後，ただちに飼育ケージに入れ，固形飼料と水を自由に与えた．新生仔が出生した日を1日目とし，7日目，14日目，21日目の肝臓をそれぞれ摘出した．ラットの乳児期は離乳する前の0～3週齢未満とした．また，日本クレア(株)よりWistar系，雄ラットも購入し，同様に固形飼料と水を自由に与えて成熟(15週齢)させ，対照として用いた．動物室は温度20℃前後，湿度60％前後を維持し，明暗サイクルは6時～18時を明，18時～6時を暗とした．なお，本実験は滋賀県立大学動物実験委員会の承認を得たものである．

2．トリプトファン-ナイアシン代謝に関わる酵素活性の測定方法

断頭によりラットをと殺後，肝臓を摘出した．直ちに肝臓を細切し，5倍量の冷却した50 mMのリン酸カリウム緩衝液(pH7.0)を加え，テフロンホモゲナイザーで均一化した．このホモジネートを酵素源とし，TDO[5]，キヌレニナーゼ(Ky-ase)[5]，3-ヒドロキシアンスラニル酸オキシゲナーゼ(3-HAO)[5]，キノリン酸ホスホリボシルトランスフェラーゼ(QPRT)[6]の活性測定を行った．

実　験　結　果

トリプトファン-ナイアシン転換率

ナイアシンは必須アミノ酸のトリプトファンから体内で合成されている．このトリプトファン-ナイアシン転換率は成人では，重量比で1/60というデータがあるが[4]，ヒト乳児のデータはなく，離乳直後(21日齢)のラットにおける実験値と成熟ラットにおける実験値の外挿から，ヒト乳児ではこの転換経路は作動しておらず，転換率は0とされている[3)7]．この推測の精度を高めるために，出産当日の1日目，7日目，14日目，21日目のラットの肝臓を取り出し，トリプトファン-ナイアシン転換経路の主要な酵素活性を測定した．その結果を表1にまとめた．

TDOはトリプトファン-ナイアシン転換経路の初発酵素である．この酵素活性が，1日目および7日目では非常に弱かった．14日，21日目と日齢とともに活性は増大し，21日目では成熟ラットの約1/2であった．測定したキヌレニナーゼ，3-ヒドロキシアンスラニル酸オキシゲナーゼ，およびキノリン酸ホスホリボシルトランスフェラーゼ活性は，出産直後の1日目でも，すでに成熟ラットの酵素活性に匹敵あるいはそれ以上の活性が検出された．

考　　察

トリプトファン-ナイアシン転換率は，年齢によって変

表1．出生後のトリプトファン-ナイアシン転換経路に関わる主要な酵素の活性変動(ラット)．

	1日目	7日目	14日目	21日目	成熟ラット
TDO	0.08 ± 0.01	0.06 ± 0.01	0.28 ± 0.04	0.48 ± 0.02	1.18 ± 0.17
Ky-ase	0.50 ± 0.01	0.41 ± 0.02	0.50 ± 0.06	1.02 ± 0.03	1.39 ± 0.18
3-HAO	528 ± 38	684 ± 25	638 ± 30	570 ± 20	627 ± 54
QPRT	1.34 ± 0.03	1.73 ± 0.03	1.06 ± 0.05	0.67 ± 0.02	0.62 ± 0.03

値は，3匹のラットの平均値±標準偏差である．単位はμmol/hr/g liverで示した．TDO = Tryptophan dioxygenase, Ky-ase = kynureninase, 3-HAO = 3-Hydroxyanthranilic acid oxygenase, QPRT = Quinolinate phosphoribosyltransferase.

動し，「第六次改定日本人の栄養所要量-食事摂取基準-」では，離乳直後のラットと成熟期のラットにおける本転換経路に関与する酵素活性の比較から，0～5カ月の乳児では0，6～11カ月の乳児では1/120としている[3]．このデータの精度を高めるために，出産当日の1日目，7日目，14日目，21日目のラットの肝臓を取り出し，トリプトファン-ナイアシン転換経路の主要な酵素活性を測定した．その結果，表1に示したように，TDOを除く他の酵素活性は，すでに出生直後においても，成熟ラットのそれらの活性とほぼ同程度の活性を有していることがはじめて明らかとなった．一方，本転換経路の初発酵素であるTDO活性が，1日目，7日目ではわずかに検出されたにすぎなかった．この事実は，ヒト乳児の初期においては，ほとんどトリプトファンからナイアシンは生合成されていないことを裏付けるものである．また，14日，21日目と日齢とともに活性は増大し，21日目では成熟ラットの約1/2であった．

この結果は，GreengardとDewey[7]が1971年に報告した結果と類似していた．今回の結果(表1)と彼らの結果[4]との間で異なる点は，今回の結果では，離乳時期に相当する出生21日後でも成熟ラットの活性の約半分であったのに対し，GreengardとDewey[4]の報告では，出生21日後で，すでに成熟ラットと同じ活性を有していたという点である．この違いは，定性的な考え方をする生化学的観点においては，重要な相違点とはなりにくいが，定量的な考え方をする栄養学的観点からは，非常に重要なことである．つまり，離乳期ですでに成熟ラットと同程度のトリプトファン-ナイアシン転換能力を有しているのか，あるいは成熟ラットの1/2程度の能力を有しているのか，という相違である．

出生1日目から約21日目までがラットの乳児期にあたることから，ラットでの転換率が，出生14日後で成熟ラットの約1/4，21日後で約1/2であったこと，および他のトリプトファン-ナイアシン転換経路に関わる酵素活性が，すでに成熟ラットとほぼ同程度まで発現していることを考えると，乳児期(0～11カ月)の後期，すなわち6～11カ月では，本転換経路が作動しはじめており，成人のトリプトファン-ナイアシン転換率の1/60の半分である1/120程度としてもさしつかえないと思われた．

結　論

ヒトの離乳期(6カ月～11カ月)のトリプトファン-ナイアシン転換率の精度を高めるために，ラットを用いて実験を行った．出産後，経日的にトリプトファン-ナイアシン転換経路の主要な酵素活性を測定した．その結果，本転換経路の初発酵素であるトリプトファン-2,3-ジオキシゲナーゼ活性が1日目，7日目ではわずかに検出されたにすぎなかった．14日目，21日目と日齢が進むとともに本酵素活性は増大し，成熟ラットの約1/2に達した．したがって，このデータをヒトにあてはめれば，乳児の離乳期すなわち6～11カ月では本転換経路は作動しはじめており，成人の半分であると算定してもさしつかえないと思われた．

謝　辞

本研究は，平成13年度～15年度の厚生労働科学研究費補助金「日本人の水溶性ビタミン必要量に関する基礎的研究」(主任研究者，柴田克己)の成果の一部である．関係各位に謝意を表する．

(平成16. 12. 22受付)

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J Nutr Sci Vitaminol, 51, 319–328, 2005

Values of Water-Soluble Vitamins in Blood and Urine of Japanese Young Men and Women Consuming a Semi-Purified Diet Based on the Japanese Dietary Reference Intakes

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(Received January 31, 2005)

Summary We investigated the levels of water-soluble vitamins except for vitamin B_6 in the blood and urine of Japanese college male ($n=10$) and female ($n=10$) students. They consumed for 7 d a semi-purified diet based on Japanese Dietary Reference Intakes to assess the Recommended Dietary Allowances (RDA) for water-soluble vitamins and to present some new normal values for blood and urine levels of water-soluble vitamins in Japanese. The blood and the 24-h urine samples were collected on the last day of the experiment and measured. The values of total vitamin B_1 in whole blood, total vitamin B_2 in whole blood, total cyanocobalamin in serum, total nicotinamide in whole blood, total pantothenic acid in whole blood, total folates in serum, total biotin in serum, and ascorbic acid in plasma were 104±17 pmol/mL (mean±SD), 216±25 pmol/mL, 0.34±0.05 pmol/mL, 59.1±5.0 nmol/mL, 2.45±0.37 nmol/mL, 15.6±4.6 pmol/mL, 8.3±0.5 pmol/mL, and 62±10 nmol/mL, respectively, in males, and 90±23, 234±18, 0.67±0.20, 61.9±6.0, 2.48±0.30, 30.2±8.6, 8.4±0.3, and 67±14, respectively, in females. There was a significant difference in the values of cyanocobalamin and total folates between men and women. The urinary excretion of vitamin B_1, vitamin B_2, cyanocobalamin, sum of the catabolic metabolites of nicotinamide, pantothenic acid, folates, biotin, and ascorbic acid were 665±114 nmol/d, 562±325 nmol/d, 93±31 pmol/d, 84±26 μmol/d, 9.3±2.3 μmol/d, 19.4±2.8 nmol/d, 83±18 pmol/d, and 148±51 μmol/d, respectively, in males, and 495±212, 580±146, 145±49, 83±19, 16.9±1.3, 22.7±2.7, 83±23, and 140±51, respectively, in females. There was a significant difference in the urinary excretion of cyanocobalamin, pantothenic acid and total folates between men and women. These values will be useful for the nutritional assessment of water-soluble vitamins for Japanese, although the examination period was short. In future, an experiment with various age groups and re-evaluation by repeated experiments will provide more accurate values.

Key Words water-soluble vitamins, blood vitamin concentrations, DRIs (Dietary Reference Intakes), humans, Japanese

In Japan, revision of the "Recommended Dietary Allowance (RDA)" has been done every 5 y since 1970 by the Ministry of Health, Labor, and Welfare. During these 3 decades, the life span of Japanese has extended and retained the first position in the world. This distinction may be attributed to the fact that the nutritional guidance and health promotion programs are excellent in Japan. In particular, in June 1999, "The 6th revision of the Japanese RDA—Dietary Reference Intakes (DRIs)—" (*1*) was laid down by the Ministry of Health, Labor, and Welfare. In this revision, the dietary reference intakes of all of the water-soluble vitamins (vitamin B_1, vitamin B_2, vitamin B_6, vitamin B_{12}, niacin, pantothenic acid, folic acid, biotin, and vitamin C) were first established. However, many RDAs were determined based on reports from foreign countries. So, we mea-

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sured the values for the blood and urine of Japanese adult male and female subjects, who ingested a diet prescribed by "The 6th revision of Japanese RDA—DRIs—" (*1*) to assess the validity for the DRIs of water-soluble vitamins.

In clinical examination, blood values are used for assessment of vitamins, but in a nutritional examination, those values would not be suitable sometimes. As a general rule, the concentrations of nutrients in the body are well controlled in healthy subjects. We think that the concentrations of water-soluble vitamins in the blood are kept almost constant in healthy adult subjects but that the urinary amounts are relatively varied, because the excess water-soluble vitamins are excreted into the urine. In other words, the blood concentrations of water-soluble vitamins would be kept constant when the subjects have ingested a more than adequate amount of nutrients, whereas the urinary excretion of water-soluble vitamins would vary, because the requirements of nutrients are different among subjects. Therefore, the measurement of urinary excretion of water-soluble vitamins should be the most reliable index for nutritional status of water-soluble vitamins. So, we investigated the water-soluble vitamin contents in the urine when subjects were given a diet based on the recommended Japanese DRIs. As far as we know, this study is the first of its kind. The values listed in the present paper will be useful as indices for evaluating the nutritional status of water-soluble vitamins.

MATERIALS AND METHODS

Subjects. Healthy Japanese college students, consisting of 10 males and 10 females, participated in the present experiment. Their ages, body weights and heights are shown in Table 1. Prior to the experiment, they had physical checkups, and their hematological and blood biochemical analyses showed normal values. This study was reviewed and approved by The Ethical Committee of The Incorporated Administrative Agency of Health and Nutrition.

Diet and experimental design. All subjects were housed in the same facility for 9 d. The experimental design is shown in Fig. 1. The 24-h urine samples were collected from the second urinary excretion on day 7 to the first one on day 8. After the volumes of the urine samples had been measured, the collected urine samples were immediately treated as described under "Analyses" to avoid destruction of water-soluble vitamins, and then stored at −20°C until needed. The blood was taken from a cubital vein at 08:30 on day 8 before breakfast, and treated immediately to avoid destruction of water-soluble vitamins, and stored at −20°C until needed.

The daily schedule was partly restricted: The lights

Table 1. Characteristics of the subjects.

Subjects	Age (y)	Height (cm)	Body weight (kg)	BMI
Male 1	19	160.2	58.50	22.79
Male 2	19	173.0	56.05	18.73
Male 3	21	176.1	70.40	22.70
Male 4	21	170.1	61.00	21.08
Male 5	21	170.5	60.95	20.97
Male 6	19	180.5	55.55	17.05
Male 7	20	166.0	57.10	20.72
Male 8	23	170.0	56.80	19.65
Male 9	20	182.0	58.70	17.72
Male 10	21	183.6	79.00	23.43
Mean	20.4	173.2	61.40	20.48
SD	1.3	7.4	7.52	2.18
SEM	0.4	2.4	2.38	0.69
Female 1	21	161.8	52.55	20.27
Female 2	22	164.8	60.90	22.42
Female 3	20	159.9	57.40	22.45
Female 4	21	162.5	51.10	19.35
Female 5	21	166.4	52.70	19.03
Female 6	21	167.7	56.50	20.09
Female 7	21	163.4	51.90	19.44
Female 8	20	158.3	51.00	20.35
Female 9	20	170.9	56.05	19.19
Female 10	20	163.6	51.35	19.19
Mean	20.7	163.9	54.15	20.08
SD	0.7	3.7	3.37	1.28
SEM	0.2	1.2	1.07	0.41

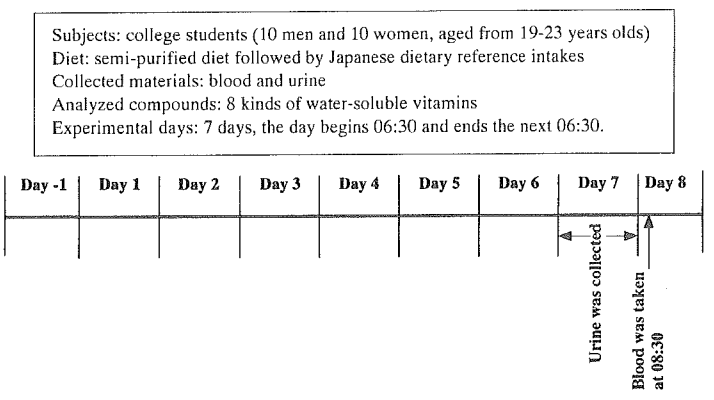

Fig. 1. Scheme of the experimental design.

Table 2. Composition of the male diet.

	(g/d)	Remarks
Wheat flour (Soft flour), first grade	315	Protein: 315 g×0.08=25.2 g. Tryptophan content was 1.1%, so 277 mg of tryptophan were supplied. Carbohydrate: 315 g×0.759=239 g Lipid: 315 g×0.017=5.4 g
Gluten	56	The gluten contained 81.6% protein, so the net protein amount was 45.7 g. Tryptophan content was 1.1%, so 503 mg of tryptophan were supplied. Total protein=70.9 g Total tryptophan=780 mg
Cornstarch	118	
Sucrose	30	Total carbohydrate=387.1 g
Fats		Total fat=50.3 g
Soybean oil	7.0	
Rapeseed oil	21.0	
Coconut oil	8.5	
Lard	8.4	
Dietary fiber		Soluble dietary fiber used, "Fibersol" was obtained from Matsutani Chemical Industry Co., Ltd. (Osaka, Japan), and insoluble dietary fiber used, Ramie powder, was obtained from Tosco Co., Ltd. (Tokyo, Japan).
Soluble	0.8	
Insoluble	14.3	
Mineral mixtures	14.6	The composition is shown below.
Total amount	592.5	Total energy=ca. 2,300 kcal=9,614 kJ

For breakfast and supper, 177.75 g of the above powder mixture was added to 110 mL of water, and mixed well, and was baked for 9 min at 250°C. The weight of the baked meal was ca. 214 g. The meal and 0.3 g of the vitamin mixture (composition shown below) were given to the subjects. For lunch, 237.0 g of the above mixture was added to 150 mL of water, and mixed well, and was baked for 10 min at 250°C. The weight of the baked meal was ca. 285 g. The meal and 0.4 g of the vitamin mixture (composition shown below) were given to the subjects.

Composition of the mineral mixture: 1,200 mg of $CaHPO_4$-$2H_2O$, 1,049 mg of $CaCO_3$, 2,124 mg of KH_2PO_4, 3,558 mg of $KHCO_3$, 2,594 mg of $MgCl_2$-$2H_2O$, 49.8 mg of $FeSO_4$-$5H_2O$, 17.6 mg of $MnSO_4$-$5H_2O$, 22.9 mg of $ZnCl_2$, 7.1 mg of $CuSO_4$-$5H_2O$, 0.2 mg of KI, and 4,000 mg of NaCl.

Composition of the vitamin mixture: 4.0 mg (2,000 IU) of retinal acetate reagent (500,000 IU/g), 2.5 μg of cholecalciferol, 4.47 mg of *dl*-α-tocopherol (7 mg was supplied from oils), 24 μg of phylloquinone (41 μg was supplied from oils), 1.2 mg of thiamin-HCl, 1.2 mg of riboflavin, 2.0 mg of pyridoxine-HCl, 2.4 μg of cyanocobalamin, 4.2 mg of nicotinamide (13.0 mg of nicotinamide was supplied from 780 mg of tryptophan in protein), 5.5 mg of calcium panthotenate, 200 μg of pteroylmonoglutamic acid, 30 μg of D(+)-biotin, 100 mg of ascorbic acid, made up to 1 g with sucrose.

were turned off at 22:00 in order to promote sleep and the subjects got up at 06:00. The breakfast time was 08:00–09:00, lunch 12:30–13:10, and dinner 18:30–19:00. They consumed a semi-purified diet based on Japanese DRIs during experiment. The composition and the amount of the semi-purified diet are shown in Table 2 for males and in Table 3 for females. The experiment was carried out from March, 1st through March, 8th, 2002 for females, and from August 27th through September 3rd, 2002 for males.

Chemicals. Wheat flour (soft flour, first grade) was obtained from Nisshin Flour Milling Inc. (Tokyo). Wheat gluten, raw cornstarch, soybean oil, 13 kinds of vitamins (see Tables 2 and 3), and minerals (see Tables 2 and 3) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Rapeseed oil was purchased from Ajinomoto Co. Ltd. (Tokyo, Japan). Coconut oil and lard were both obtained from Clea Japan (Tokyo, Japan). "Fibersol," used as a soluble dietary fiber, was obtained from Matsutani Chemical Industries (Osaka, Japan) and "Ramie powder," used as an insoluble dietary fiber, was from Tosco (Tokyo, Japan).

Thiamin hydrochloride ($C_{12}H_{17}ClN_4OS$-HCl=337.27), riboflavin ($C_{17}H_{20}N_4O_6$=376.37), cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$=1355.40), nicotinamide (Nam; $C_6H_6N_2O$=122.13), calcium pantothenate ($C_{18}H_{32}N_2O_{10}$-Ca=476.54), folic acid ($C_{19}H_{19}N_7O_6$=441.40), D(+)-biotin ($C_{10}H_{16}N_2O_3S$=244.31), and L(+)-ascorbic acid ($C_6H_8O_6$=176.13) were purchased from Wako Pure Chemical Industries. N^1-Methylnicotinamide (MNA) chloride ($C_7H_9N_2O$-HCl=159.61) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). N^1-Methyl-2-pyridone-5-carboxamide (2-Py, $C_7H_8N_2O_2$=152.15) and N^1-methyl-4-pyridone-3-carboxamide (4-Py, $C_7H_8N_2O_2$=152.15) were synthesized by the methods of Pullman and Colowick (*2*) and Shibata et al. (*3*), respectively.

All other chemicals used were of the highest purity available from commercial sources.

Analyses

Blood and urine

Vitamin B_1 (thiamin): The concentrations of total vitamin B_1 in whole blood and urine were measured by the HPLC-post labeled fluorescence method of Kimura et al. (*4*).

Vitamin B_2 (riboflavin): The concentration of total vitamin B_2 in whole blood was determined by the